Modeling GPCR-induced biased signaling Towards a system biology definition of drugs selectivity

Romain Yvinec

Modeling GPCR-induced biased signaling

Towards a system biology definition of drugs selectivity

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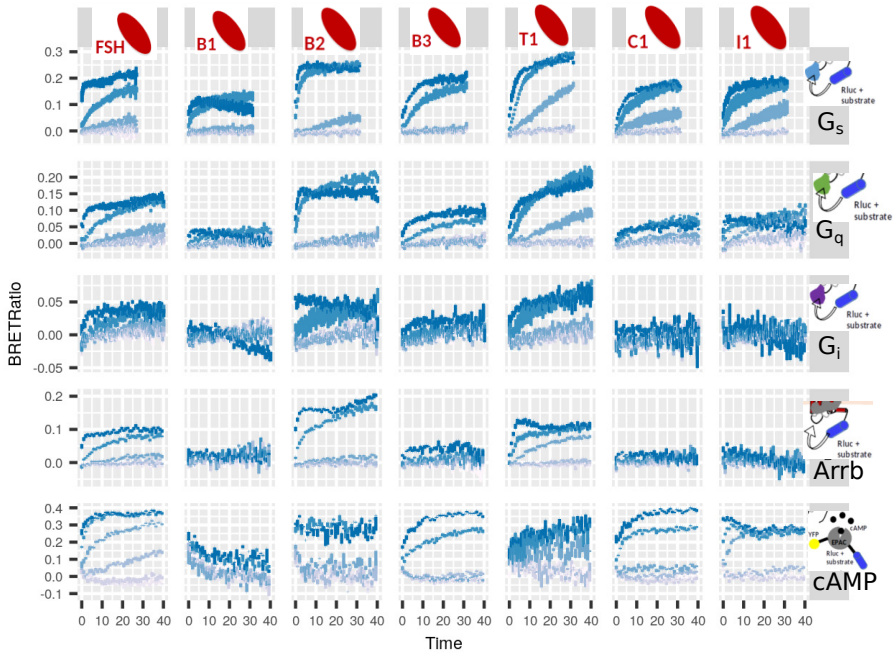
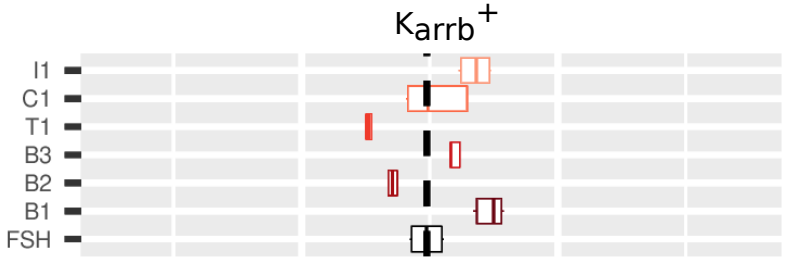
BIOS, INRAE Tours

Take-home Message : use Maths !



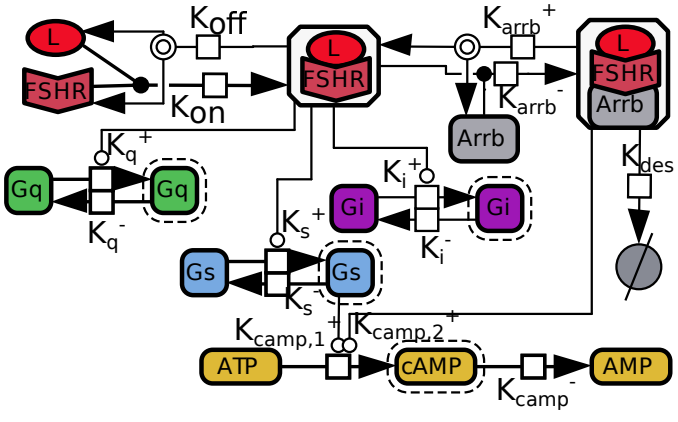


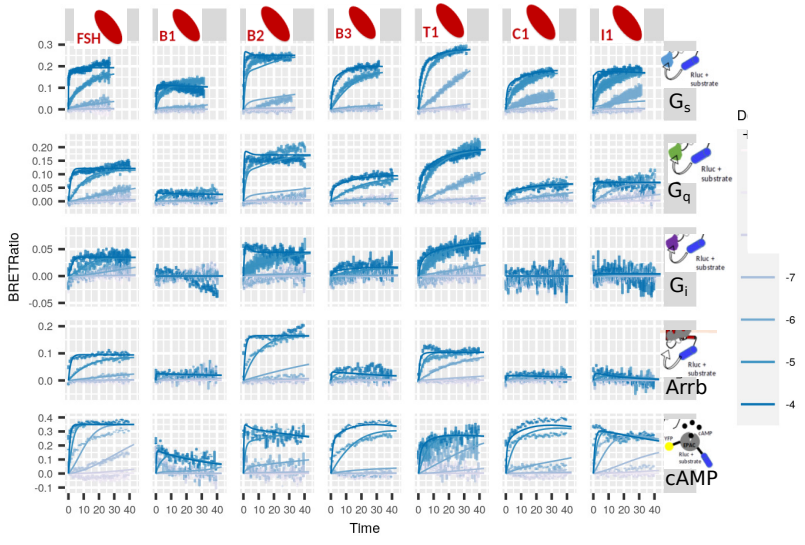
Kinetic pathway modeling to

Fully exploit kinetic data

Give mechanistic insight of pharmacological Ligand properties

$$\frac{d}{dt}[LR] = k_{ON}[L][R] - k_{OFF}[LR]$$

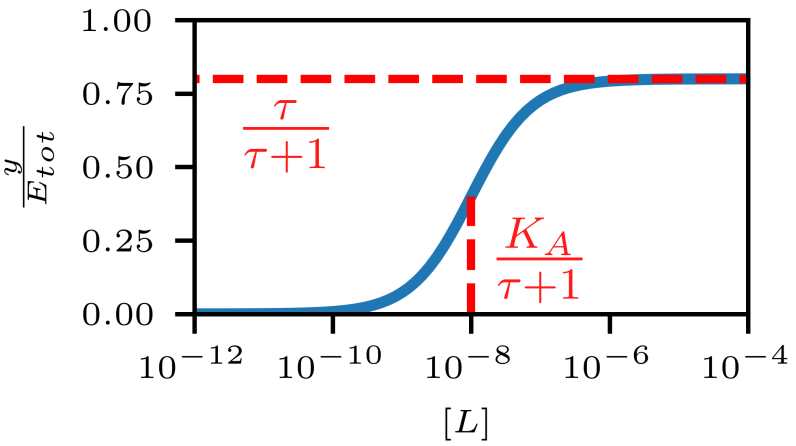


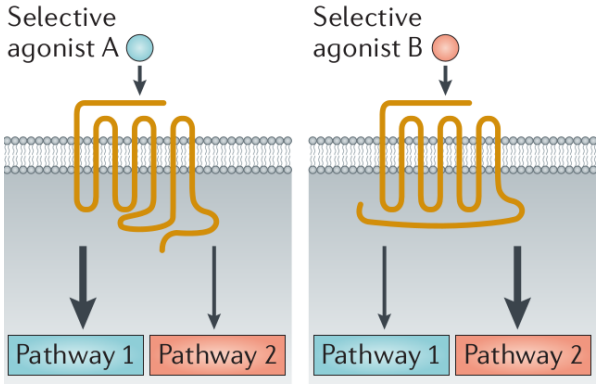


Biased signaling & standard quantification

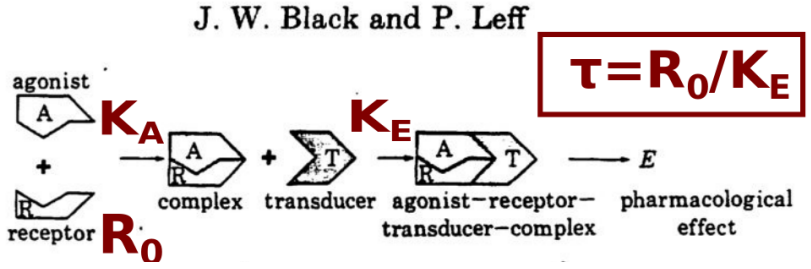
Operational model

$$y = E_{tot} \frac{\tau[L]}{K_A + (\tau + 1)[L]} .$$





Kenakin and Christopoulos, *Nat. Rev. Drug Discov.* (2013)



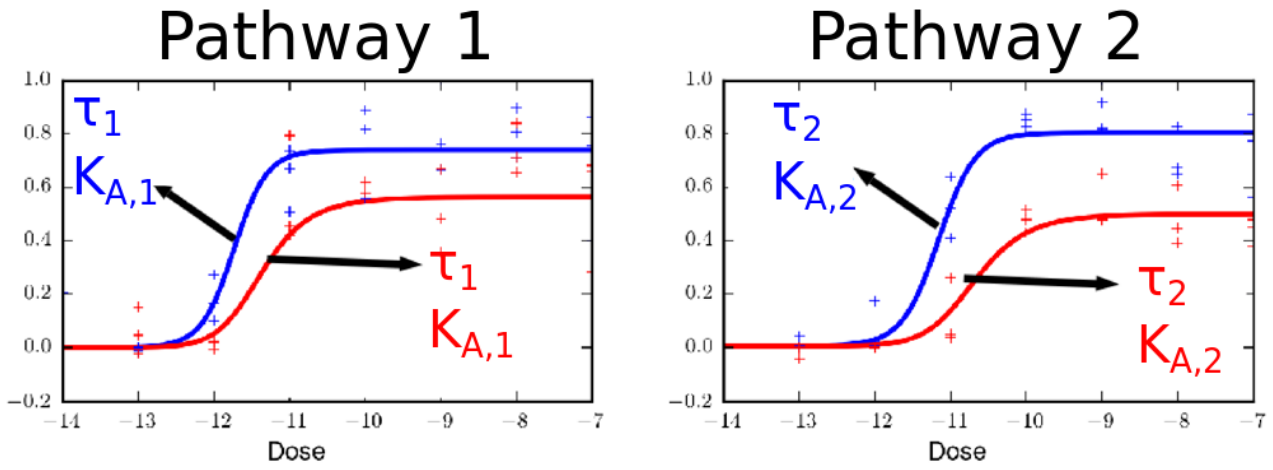
Equilibrium operational model

Black and Leff, *Proc. R. Soc. Lond. B* (1983) .

$\Rightarrow$ **Transduction coefficient** :

$$log(R) := \log\left(\frac{\tau}{K_A}\right) = \frac{\text{Observed Efficacy}}{\text{Observed Potency}}$$

Biased signaling & standard quantification



$\Rightarrow$ **Bias** : $\Delta\Delta \log(\tau/Ka) =$

$(\log(\tau_1/K_{A,1}) - \log(\tau_2/K_{A,2})) - (\log(\tau_1/K_{A,1}) - \log(\tau_2/K_{A,2}))$

Identifiability issue : Zhu et al., BJP 175 :1654–1668, 2018

Pro

- ⋆ Mechanistic basis
- ⋆ Generic and widely applicable
- ⋆ A single parameter

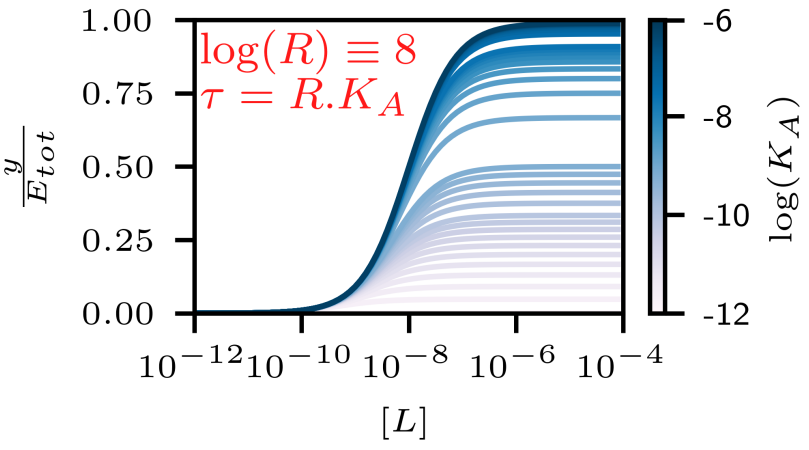
Cons

- ⋆ No kinetic
- ⋆ Inconsistent within a transduction pathway
- ⋆ A single parameter

Biased signaling & standard quantification

- The transduction coefficient is not always meaningful
- Identifiability rely on correct estimation of observed Potency

Scanning over fixed R



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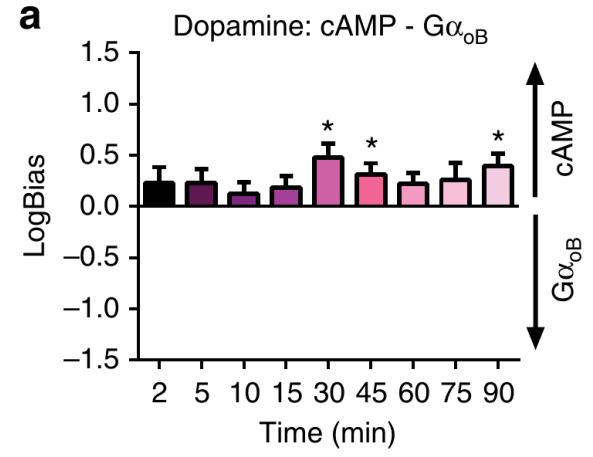
Time-dependent bias ?

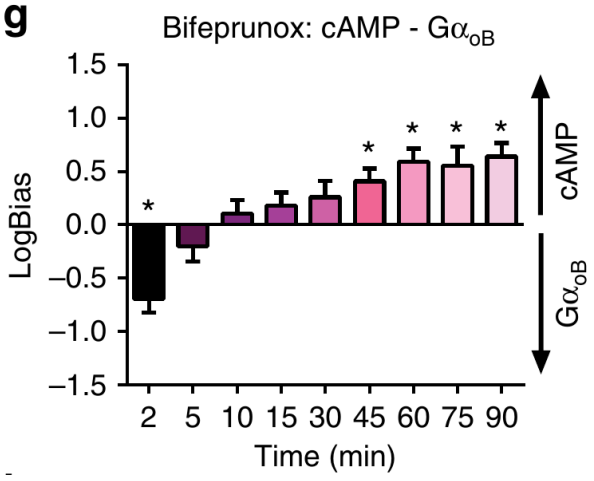
The role of kinetic context in apparent biased agonism at GPCRs

Carmen Klein Herenbrink[1], David A. Sykes[2], Prashant Donthamsetti[3,4], Meritxell Canals[1], Thomas Coudrat[1], Jeremy Shonberg[5], Peter J. Scammells[5], Ben Capuano[5], Patrick M. Sexton[1], Steven J. Charlton[2], Jonathan A. Javitch[3,4,6], Arthur Christopoulos[1] & J Robert Lane[1]

- Bias value may change according to the response time after stimulation.
- Kinetic explanation : Ligands with a slow binding kinetics may have changing bias value according to time.

Klein Herenbrink et al., *Nat. Commun* (2016)





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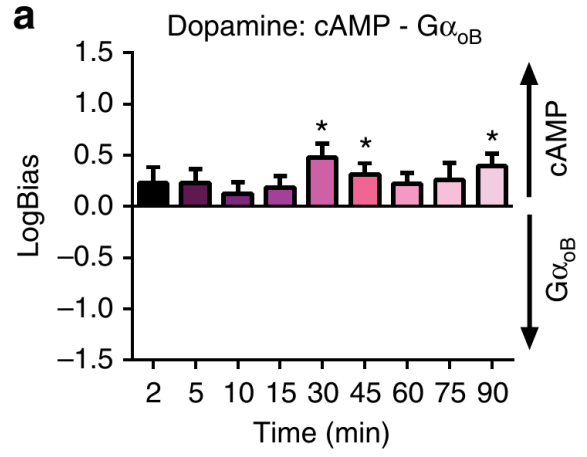
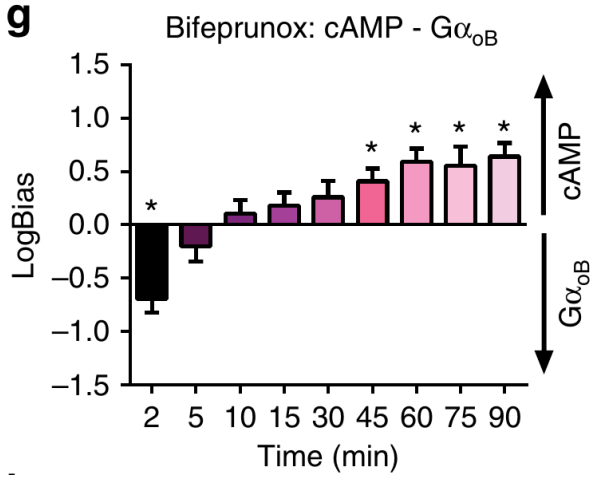
- Kinetic explanation : Ligands with a slow binding kinetics may have changing bias value according to time.

⇒ **We need to take into account dynamic patterns in bias quantification**





Motivations and Case study

Use reaction network modeling *(kinetic pathway)* to

- Fully exploit kinetic data
- Give more mechanistic insight of signaling bias
- Develop a parsimonious and statistically significant framework to characterize pharmacological ligand properties

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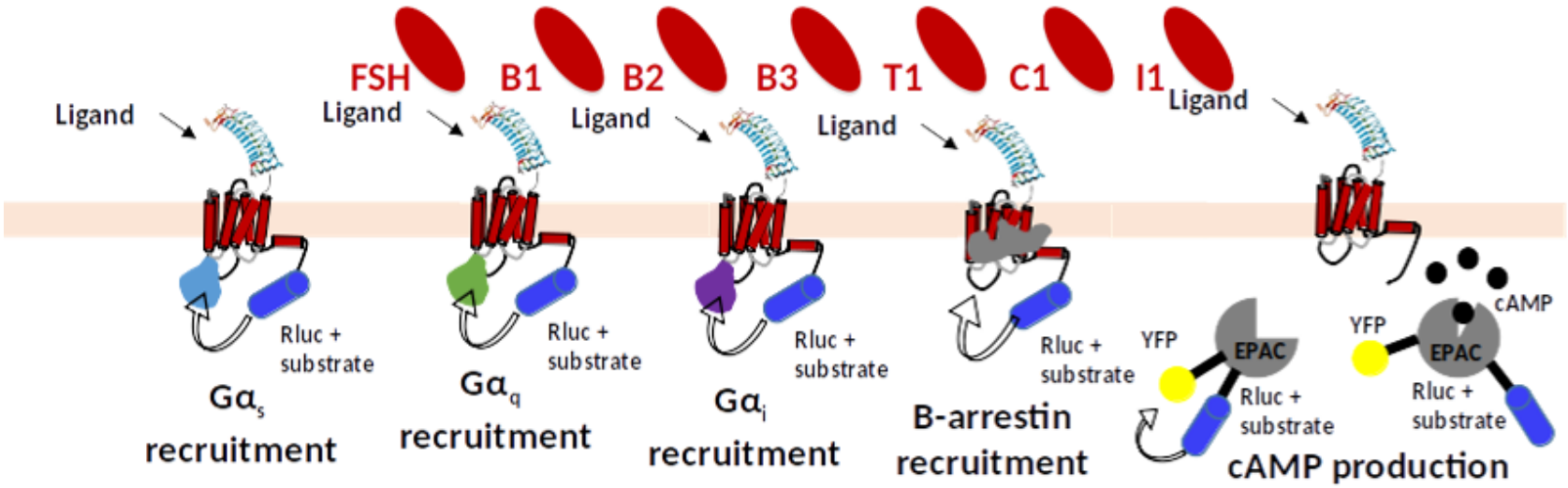
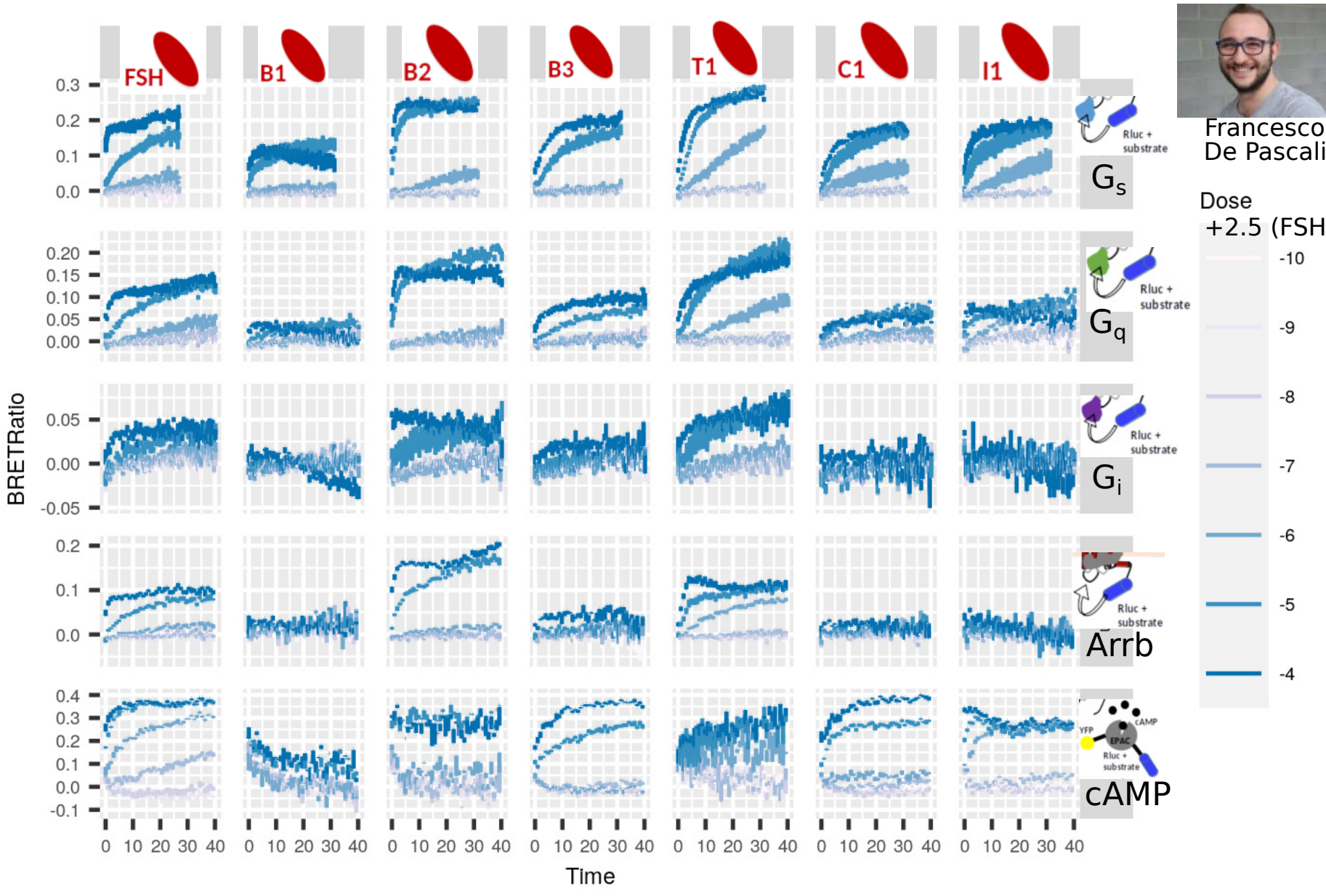
Case study on FSHR

⋆ 5 BRET sensors : NES-Venus mG, yPET- β-arrestin 2, Camyel

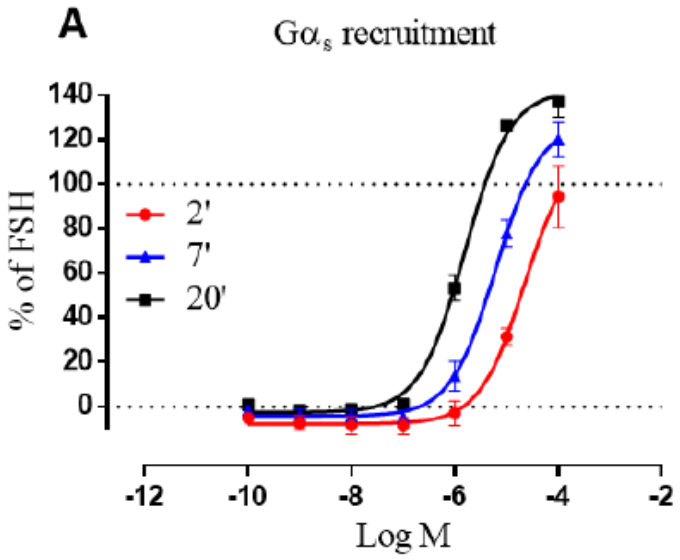
⋆ FSH + 6 LMW compounds (Benzamides, Thiazolidinone, Chromenopyrazole, Imidazole) (TocopheRx, Burlington, VT, USA).

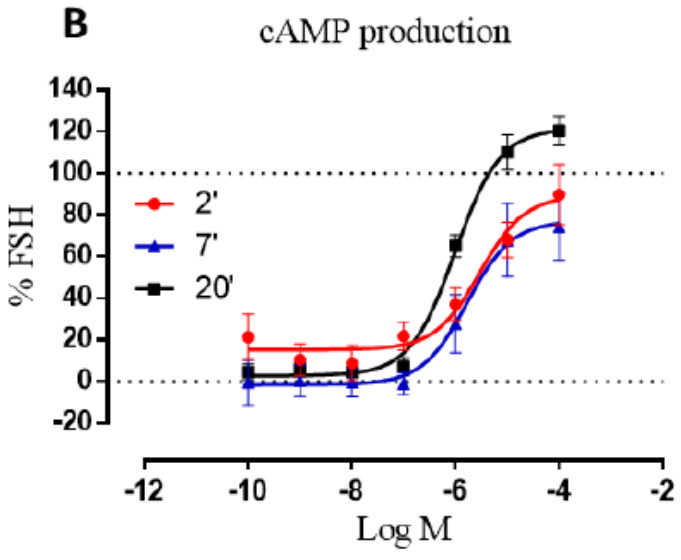




Francesco De Pascali

Potency and Efficacy (and bias) are time point dependent



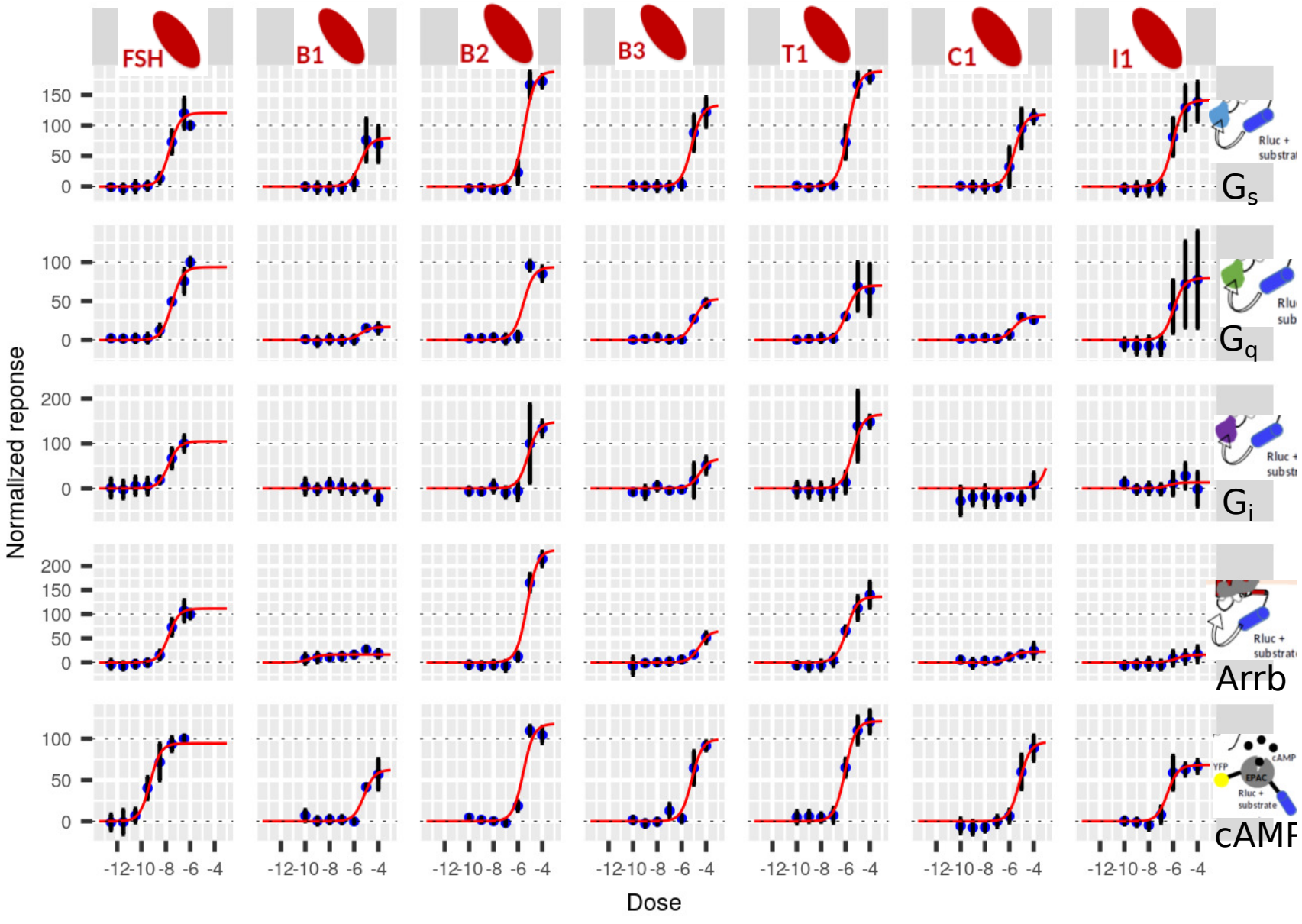


C

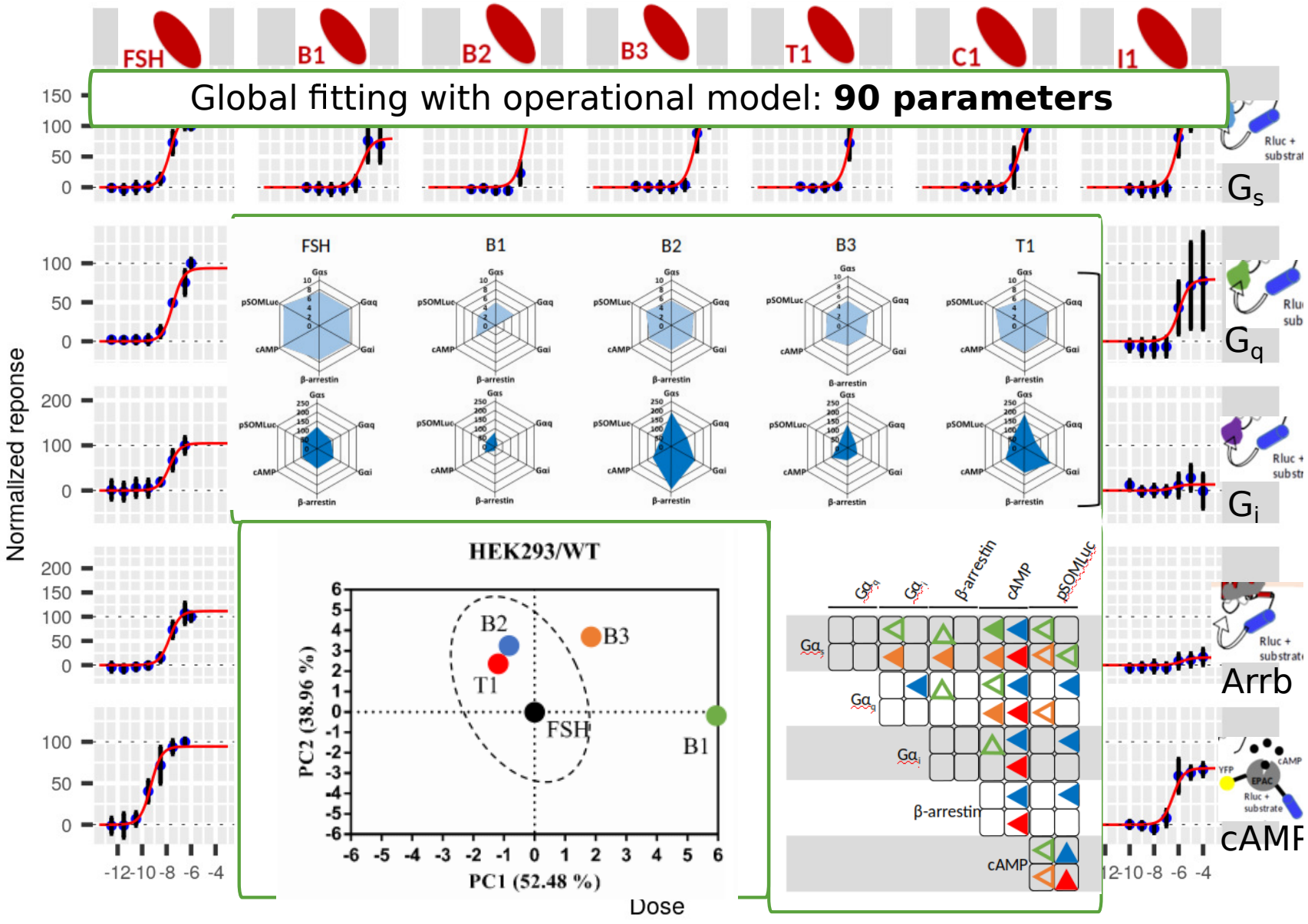
	$G\alpha_s$ recruitment			cAMP production		
	Emax (% of max average) ± SEM (n=6)					
Ligand	2'	7'	20'	2'	7'	20'
FSH	117.1 ± 10.6	120.2 ± 11.5	104.1 ± 8.3	102.7 ± 1.8	100.6 ± 2.1	98.2 ± 1.3
T1	116.9 ± 12.2	127.7 ± 5.8	129.1 ± 2.7	89.5 ± 9.5	77.3 ± 10.1	121.6 ± 4.3
	Ligand pEC_{50} ± SEM (n=6)					
T1	4.7 ± 0.1	5.2 ± 0.1	5.8 ± 0.1	5.5 ± 0.3	5.7 ± 0.3	6.0 ± 0.1

*** (FSH 104.1 ± 8.3 vs T1 129.1 ± 2.7)
* (FSH 98.2 ± 1.3 vs T1 121.6 ± 4.3)
**** (4.7 ± 0.1 vs 5.2 ± 0.1)
**** (5.2 ± 0.1 vs 5.8 ± 0.1)
**** (4.7 ± 0.1 vs 5.8 ± 0.1)
* (5.5 ± 0.3 vs 6.0 ± 0.1)

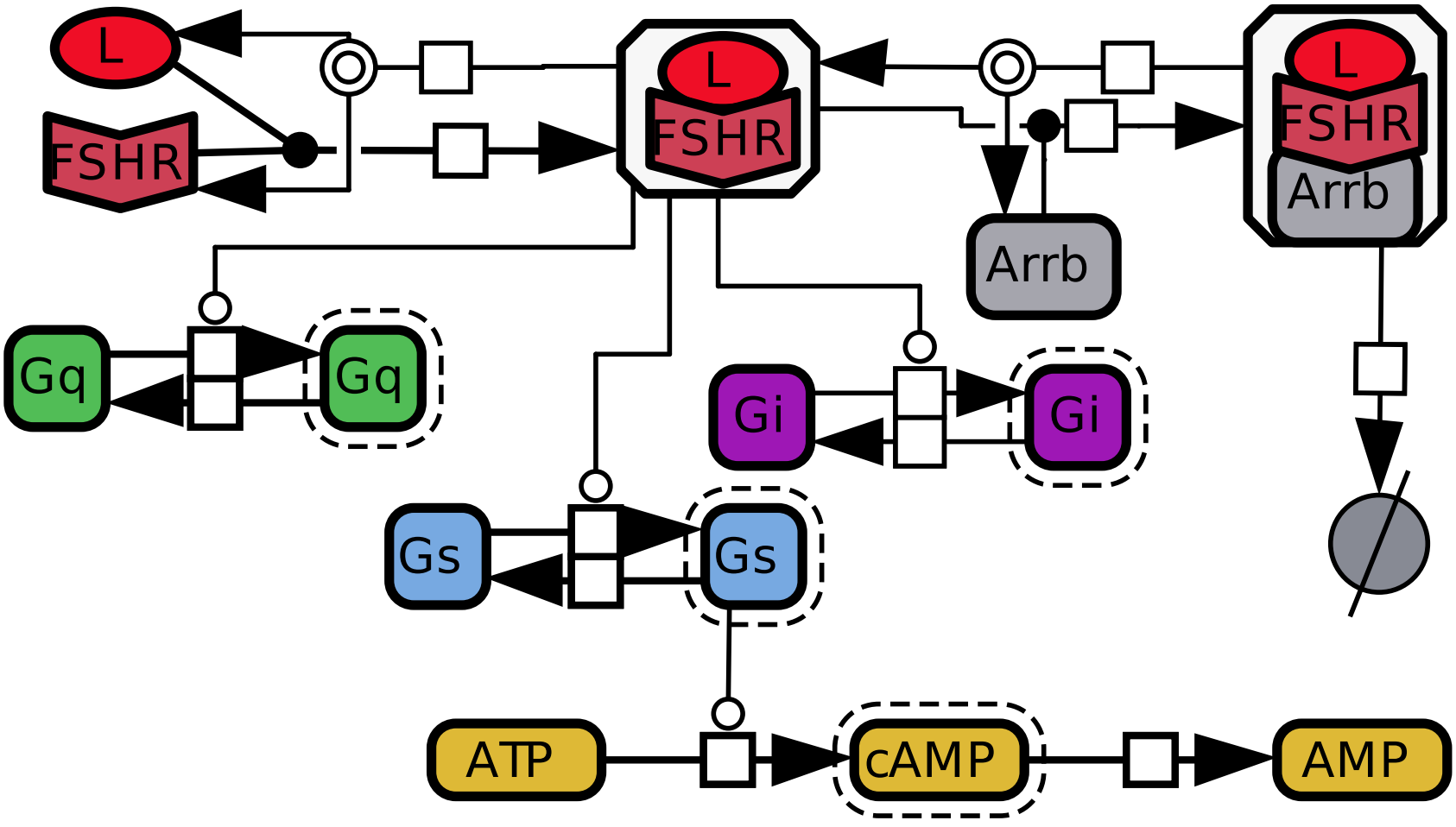
Operational model with A.U.C



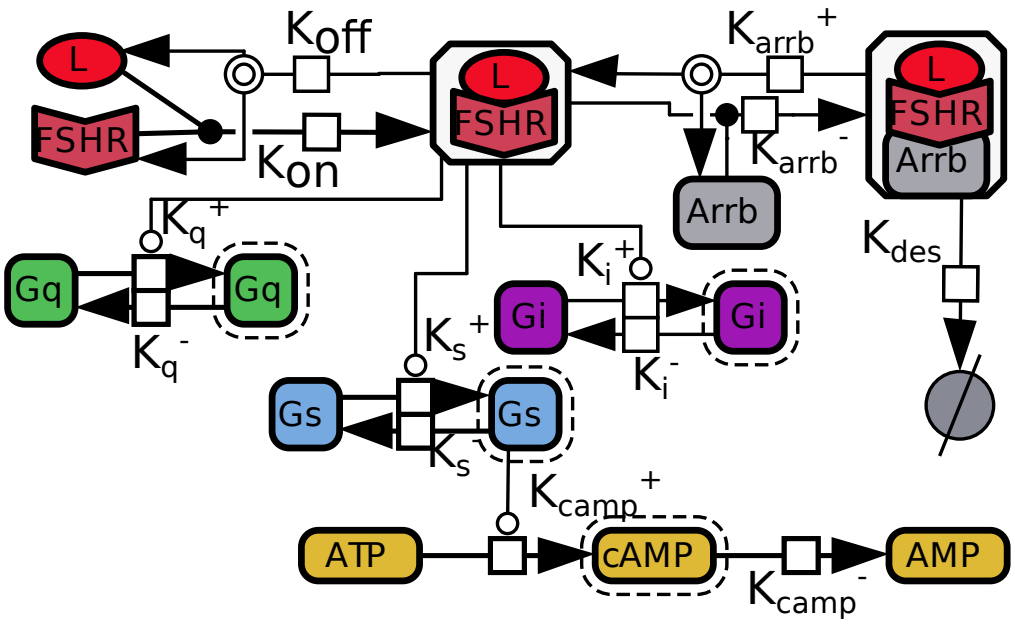
Operational model with A.U.C



Reaction network : multiple Pathways modeling

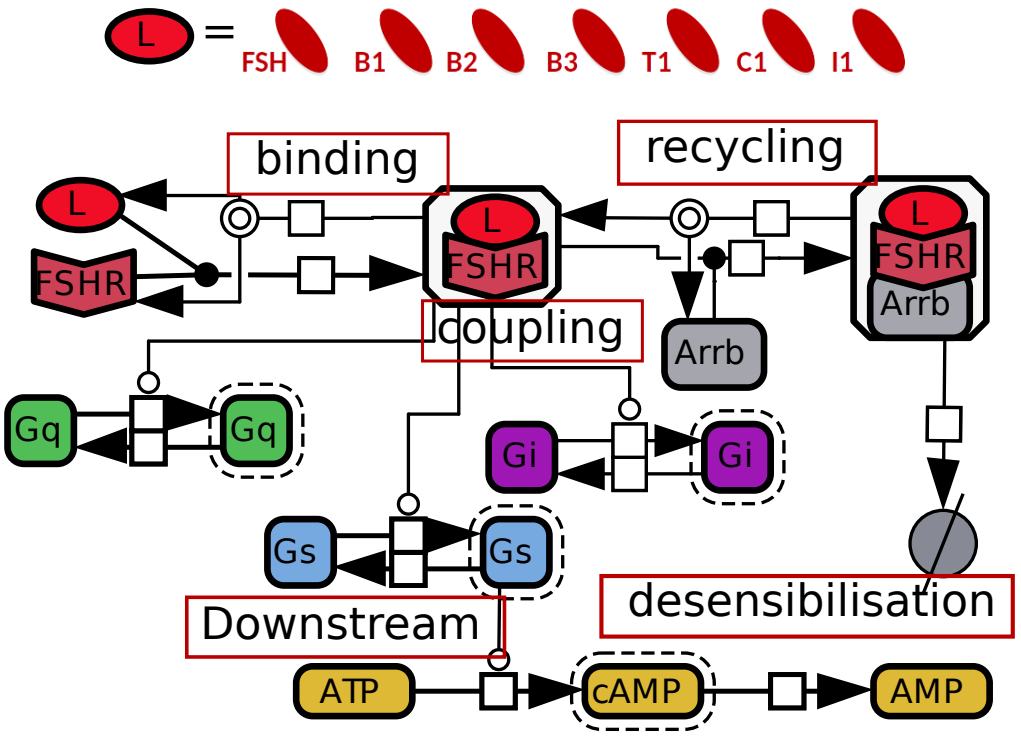


Generate all pathways at once



- Dynamic reaction networks (ODE) keep track of concentration of each molecule along time.
- Parameters : initial quantity of molecules and kinetic rates (13).

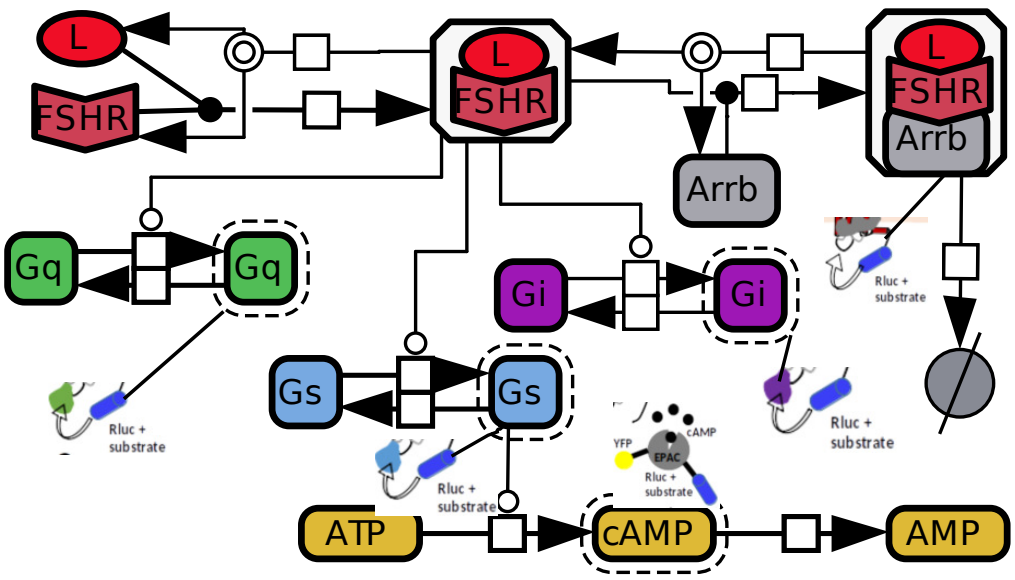
Mechanistic link with data



We hypothesize that

- Kinetic rate values reflects pharmacological ligand properties.
- Measurements are performed in a same cellular context.
- Measurements are proportional to concentration of molecules.

Mechanistic link with data



We hypothesize that

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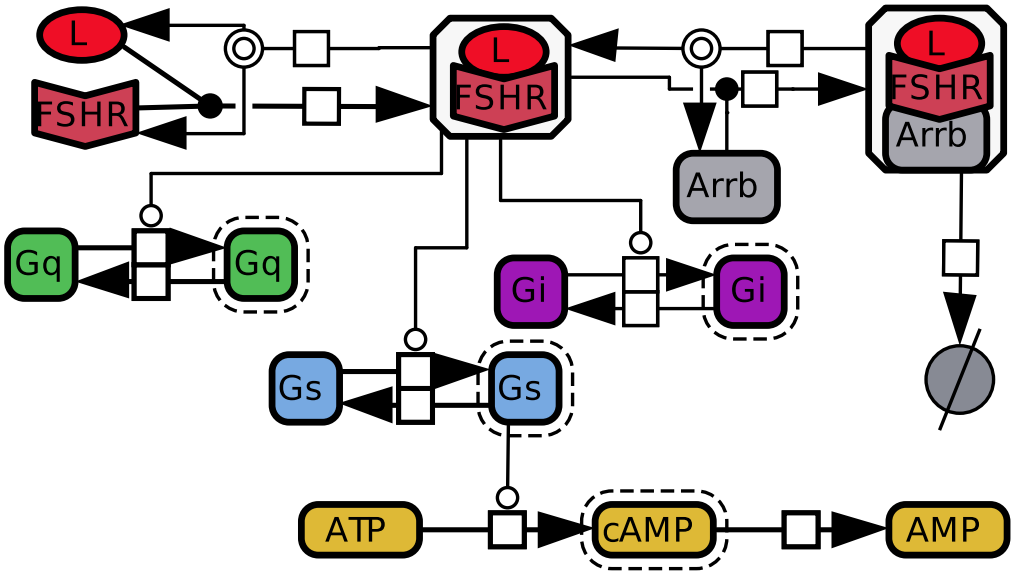
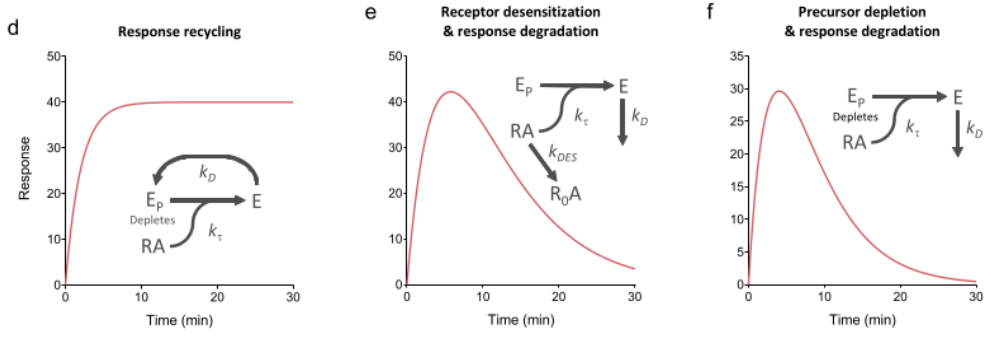
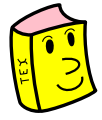
Signaling profile diversity



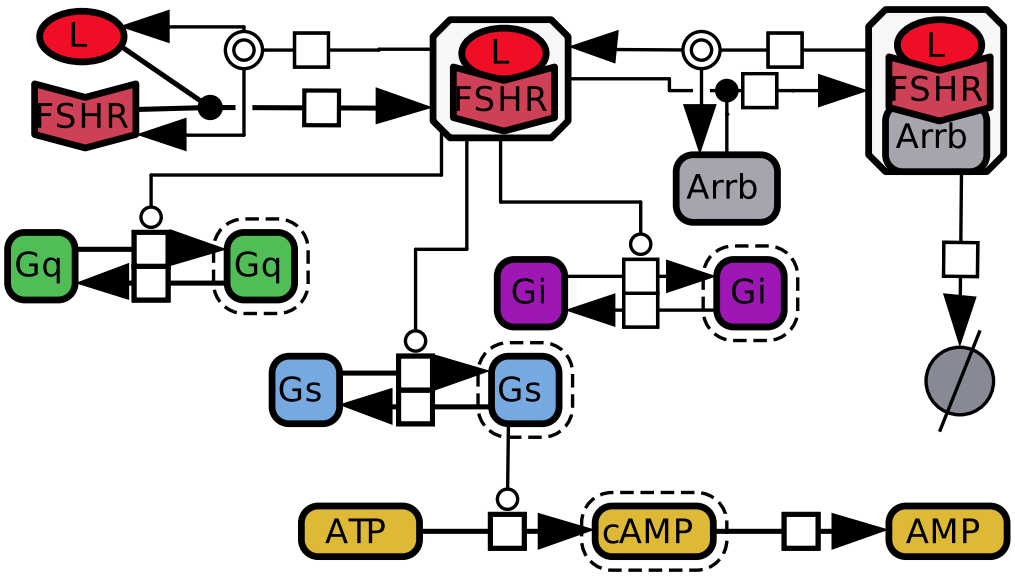


- The model is "minimal" *(model selection criteria)*
- We generalize recent attempts to define a "kinetic operational model" (Watch Nicola Dijon's flash presentation)



Hoare et al., Analyzing kinetic signaling data for G-protein-coupled receptors, Scientific Reports 2020

Global fitting enforcing sparsity

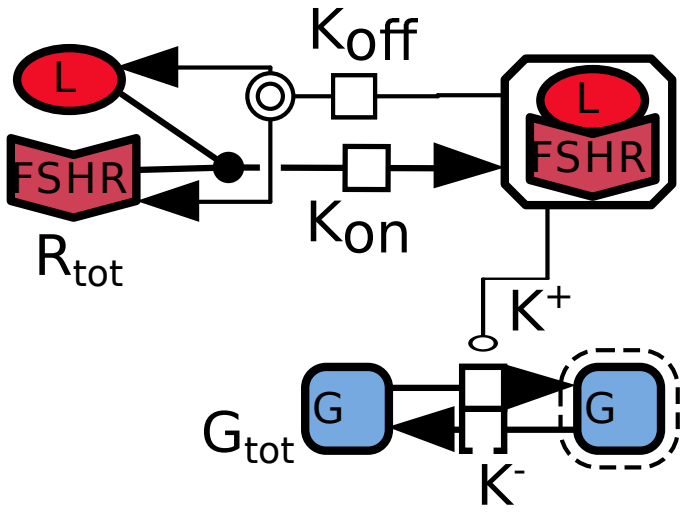


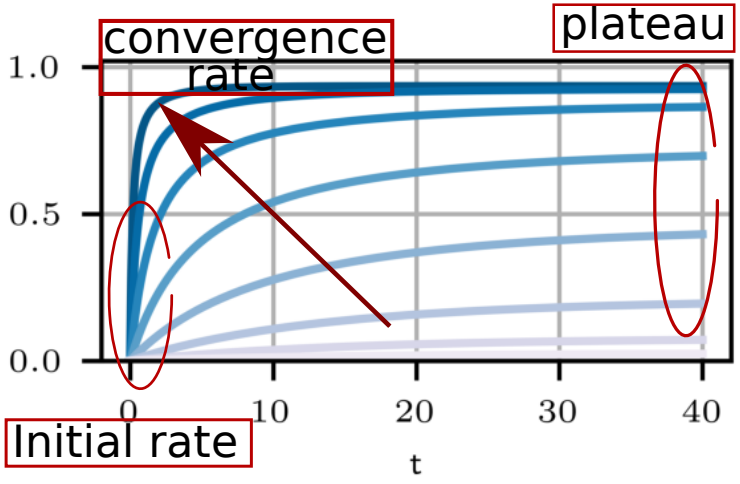
- Our method is a global fitting approach (all pathways, all ligand).
- We enforce Ligand specific parameters through penalization.

Raue et al., Data2Dynamics : a modeling environment tailored to parameter estimation in dynamical systems, Bioinformatics 2015

Steiert et al., L1 regularization facilitates detection of cell type-specific parameters in dynamical systems, Bioinformatics 2016

Can we really infer parameter from kinetic data ?





- Initial rate

$$\frac{1}{2} R_{tot} G_{tot} k_{on} k^{+} [L] t^2$$

- Equilibrium

$$\frac{R_{tot} G_{tot} [L]}{K_A + (R_{tot} + K_E)[L]}$$

- Convergence rate

$$k^{+} \frac{R_{tot}[L]}{K_A + [L]} + k^{-}$$

$$K_A = \frac{K_{off}}{K_{on}} , K_E = \frac{K^{-}}{K^{+}}$$

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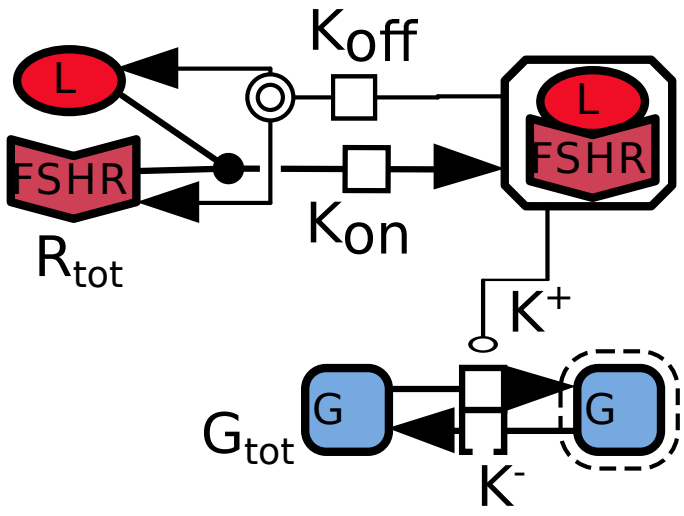
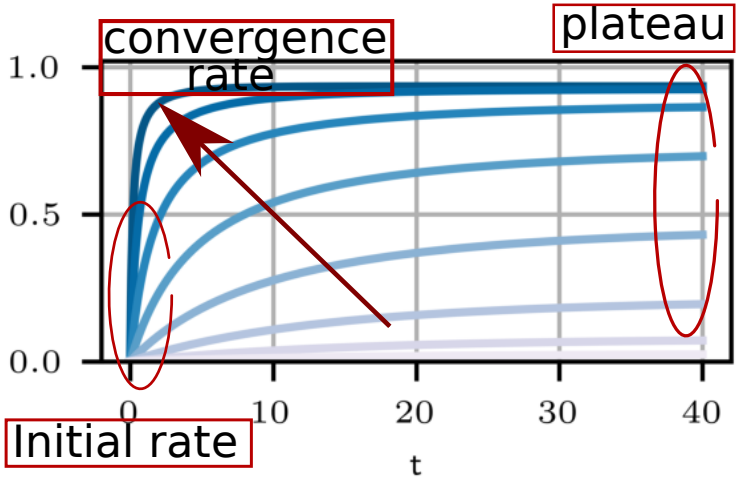
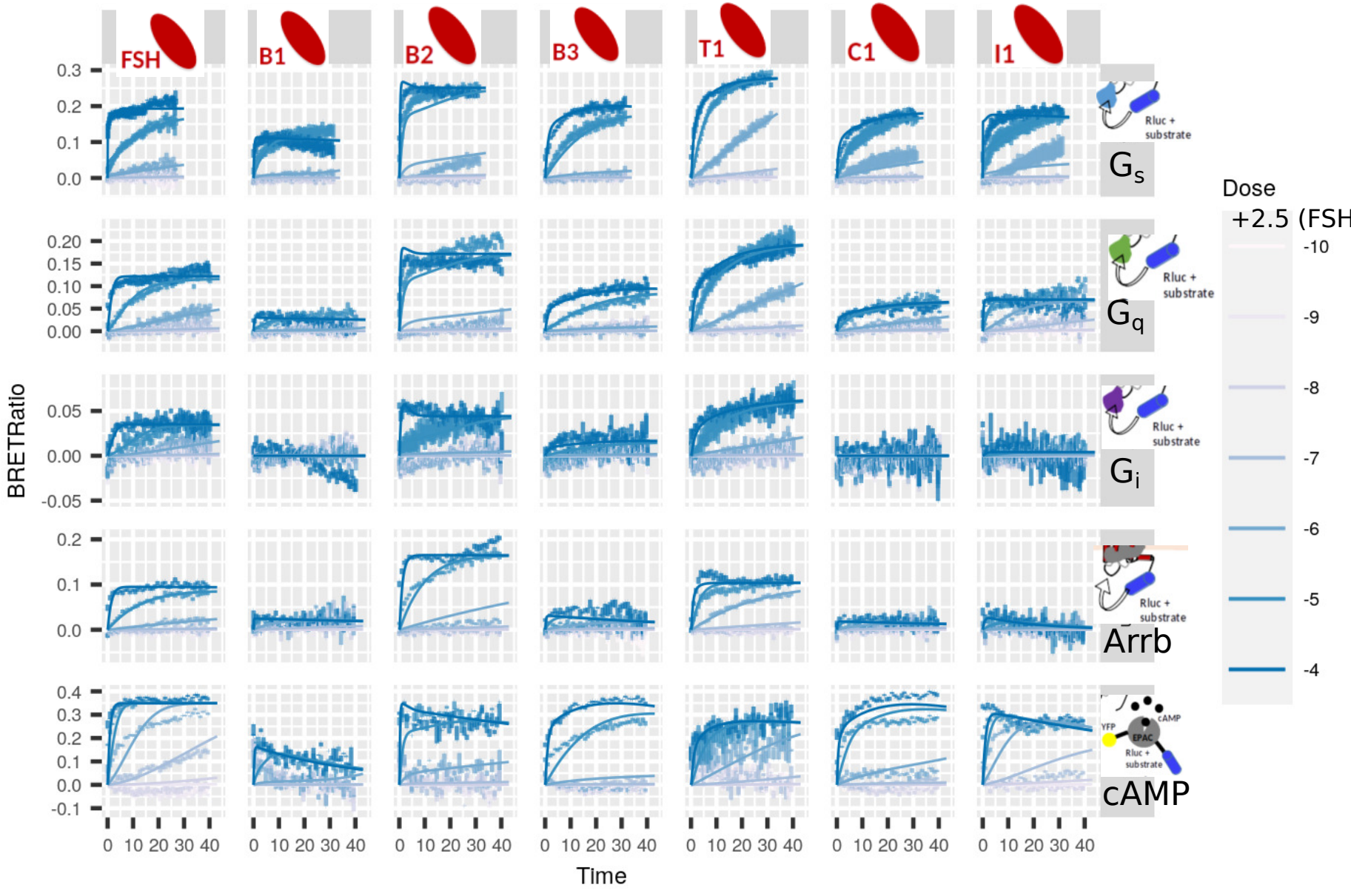




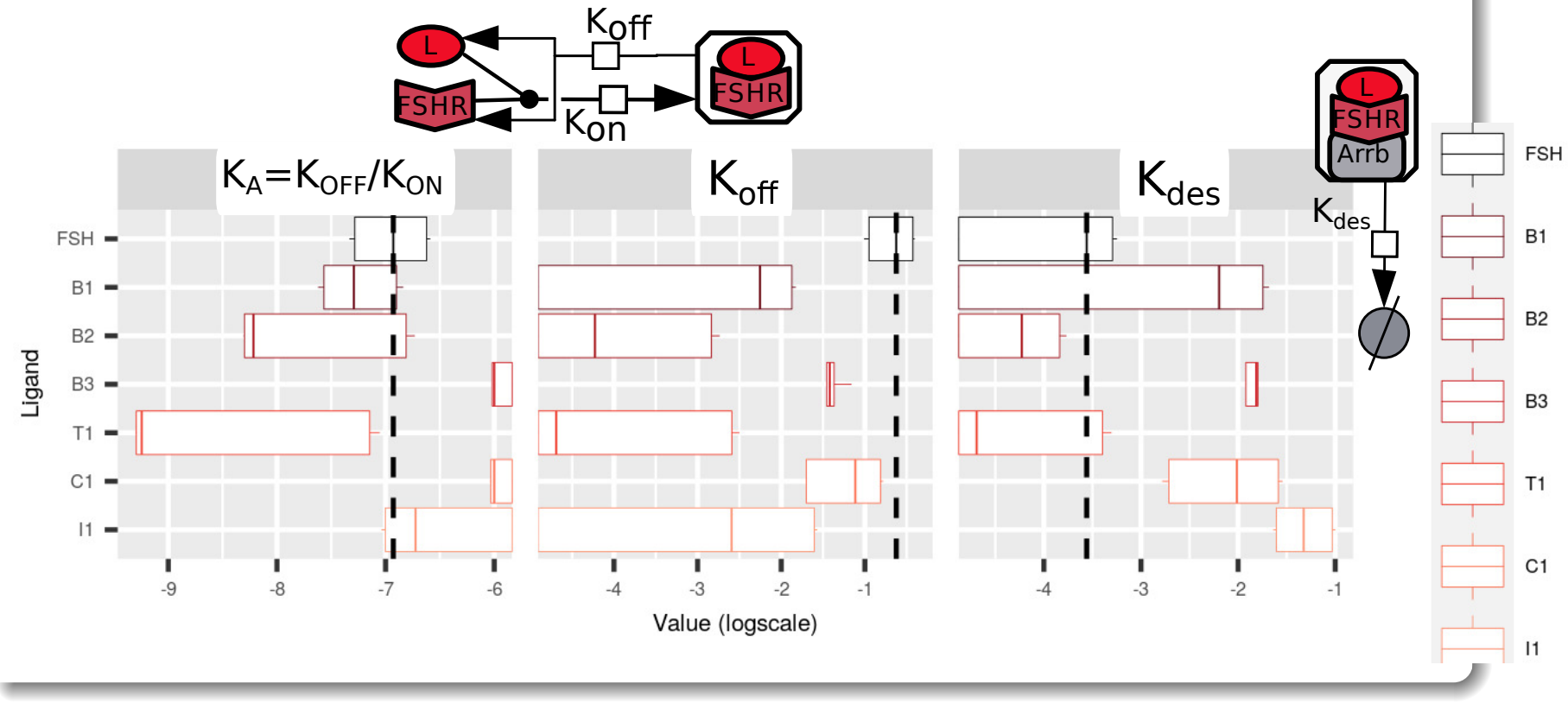
- Initial rate $\frac{1}{2} R_{tot} G_{tot} k_{on} k^+ [L] t^2$
- Equilibrium $\frac{R_{tot} G_{tot} [L]}{K_A + (R_{tot} + K_E)[L]}$
- Convergence rate $k^+ \frac{R_{tot}[L]}{K_A + [L]} + k^-$

→ In practice the global fitting improves parameter identifiability.

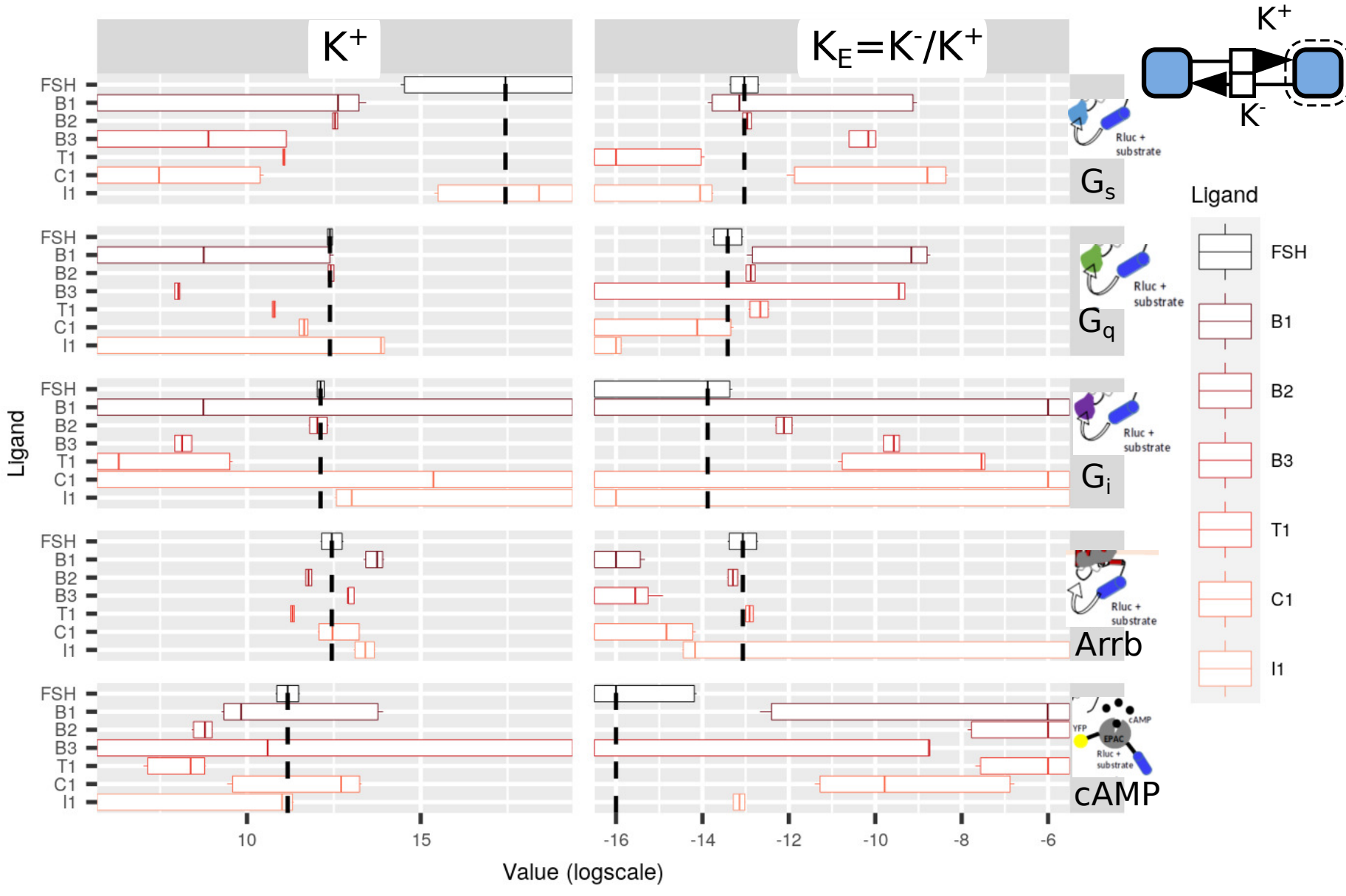
→ Low doses and long time signal are important.

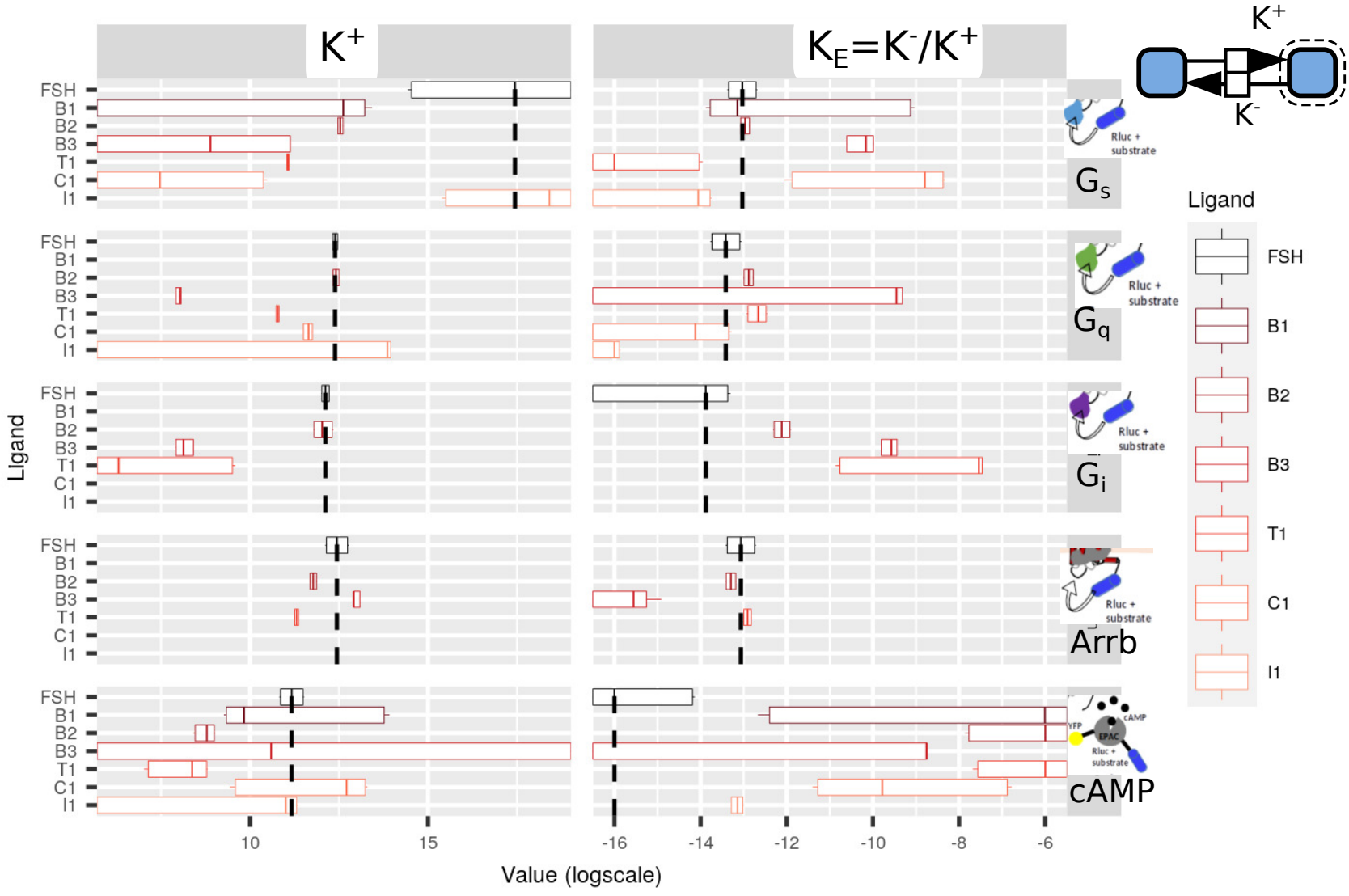

FSH
B1
B2
B3
T1
C1
I1
Rluc + substrate
G_s
G_q
G_i
Arrb
YFP
cAMP
EPAC
cAMP
BRETRatio
Time
Dose
+2.5 (FSH
-10
-9
-8
-7
-6
-5
-4

Inferring Binding and Desensibilisation constants

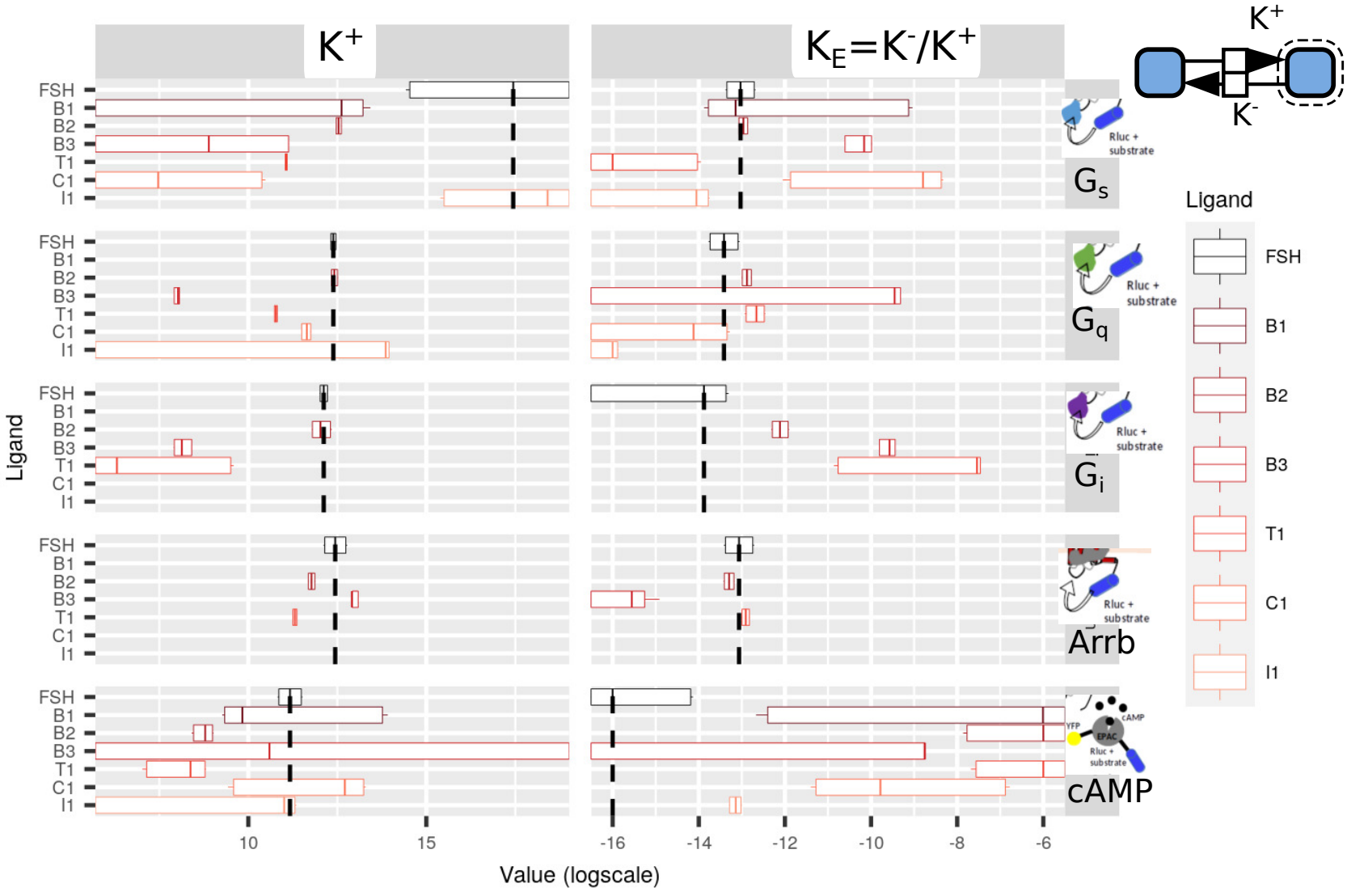


$\Rightarrow$ We can infer K_A (with potentially asymmetric confidence intervals).

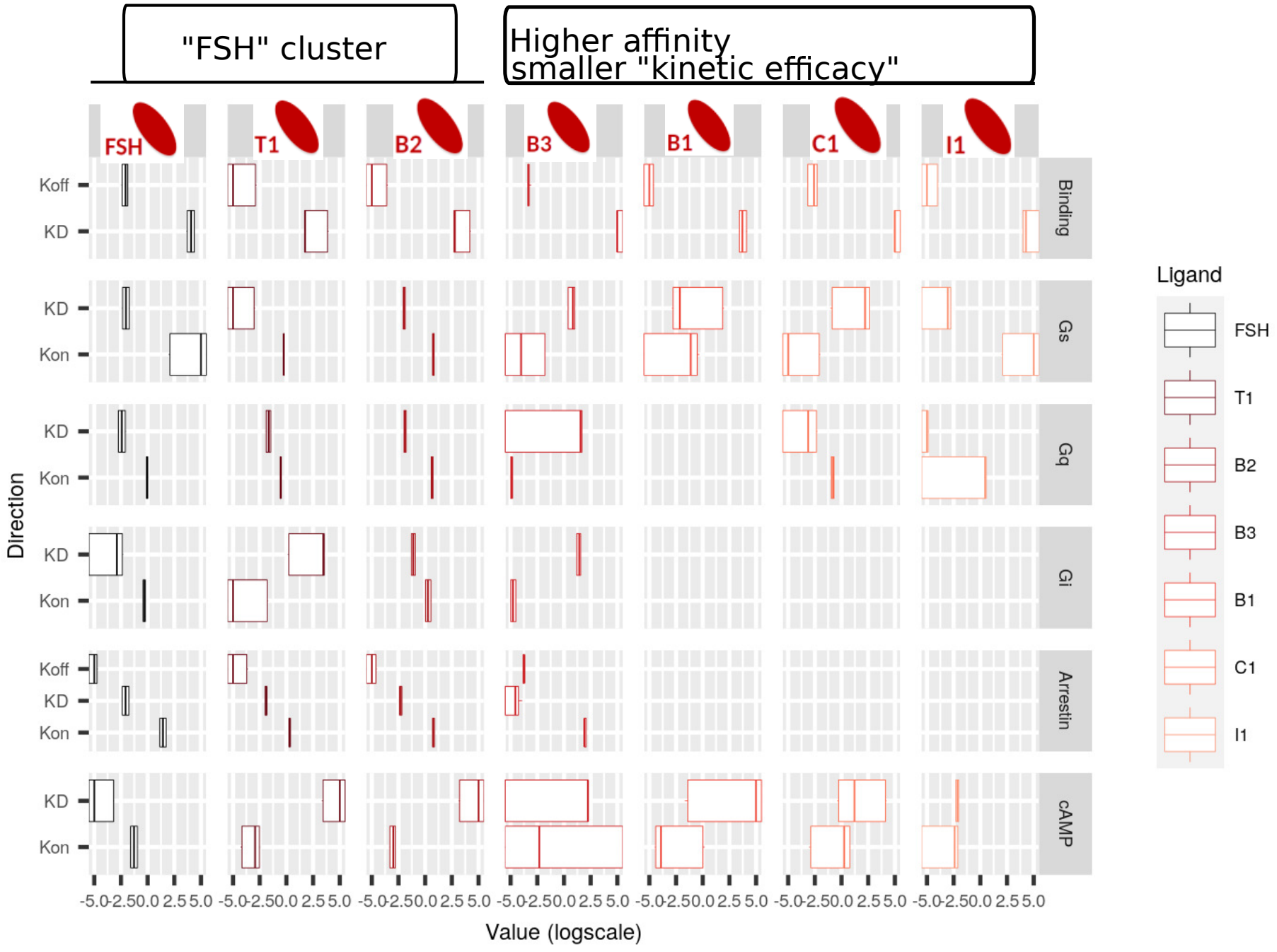

K+
KE=K-/K+
K+
K-
Ligand
FSH
B1
B2
B3
T1
C1
I1
Rluc + substrate
Gs
Gq
Gi
Arrb
cAMP
YFP
EPAC
cAMP
10
15
-16
-14
-12
-10
-8
-6
Value (logscale)

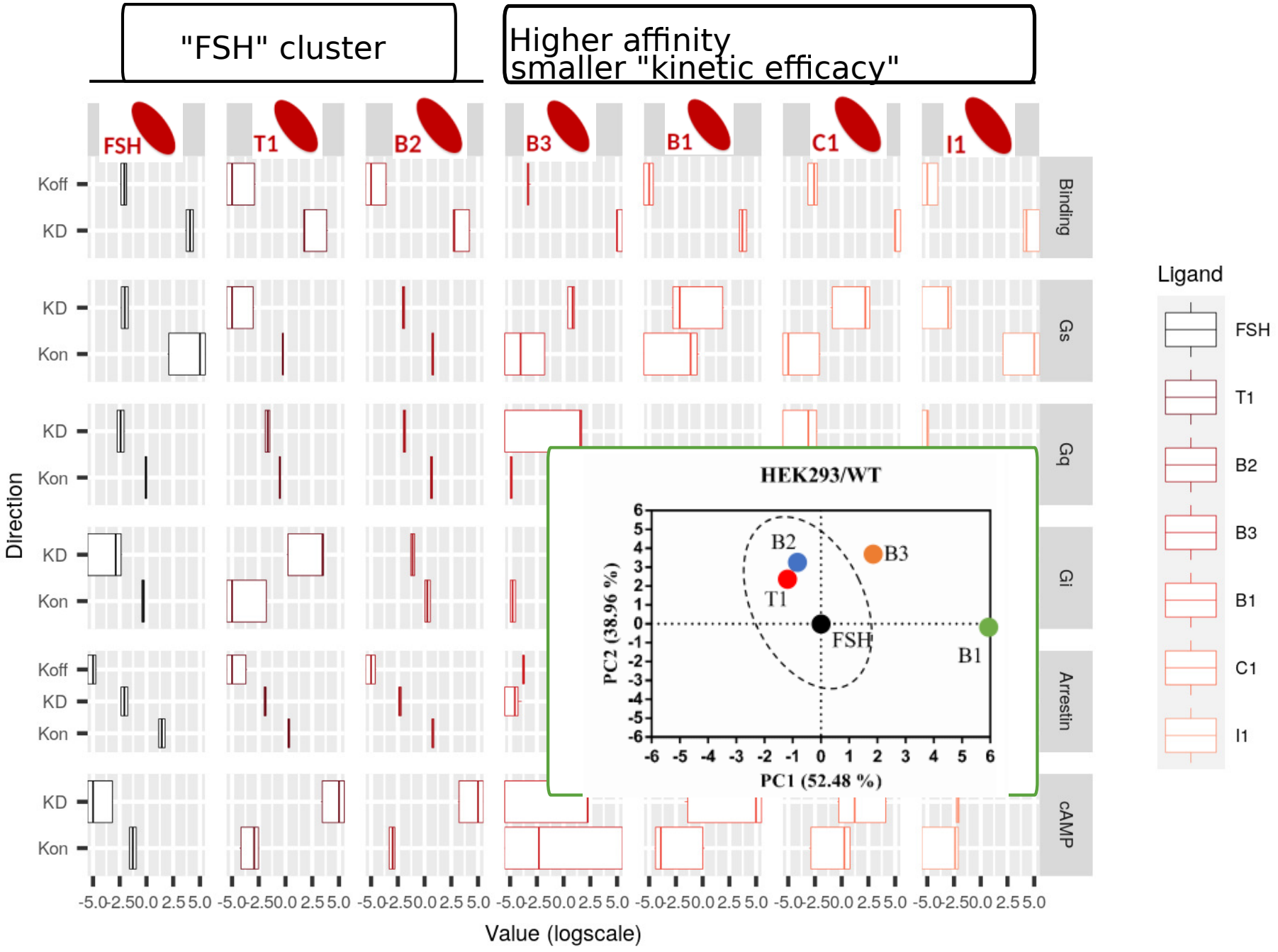


⇒ Non-identifiable parameters are consistent with no signals from data.

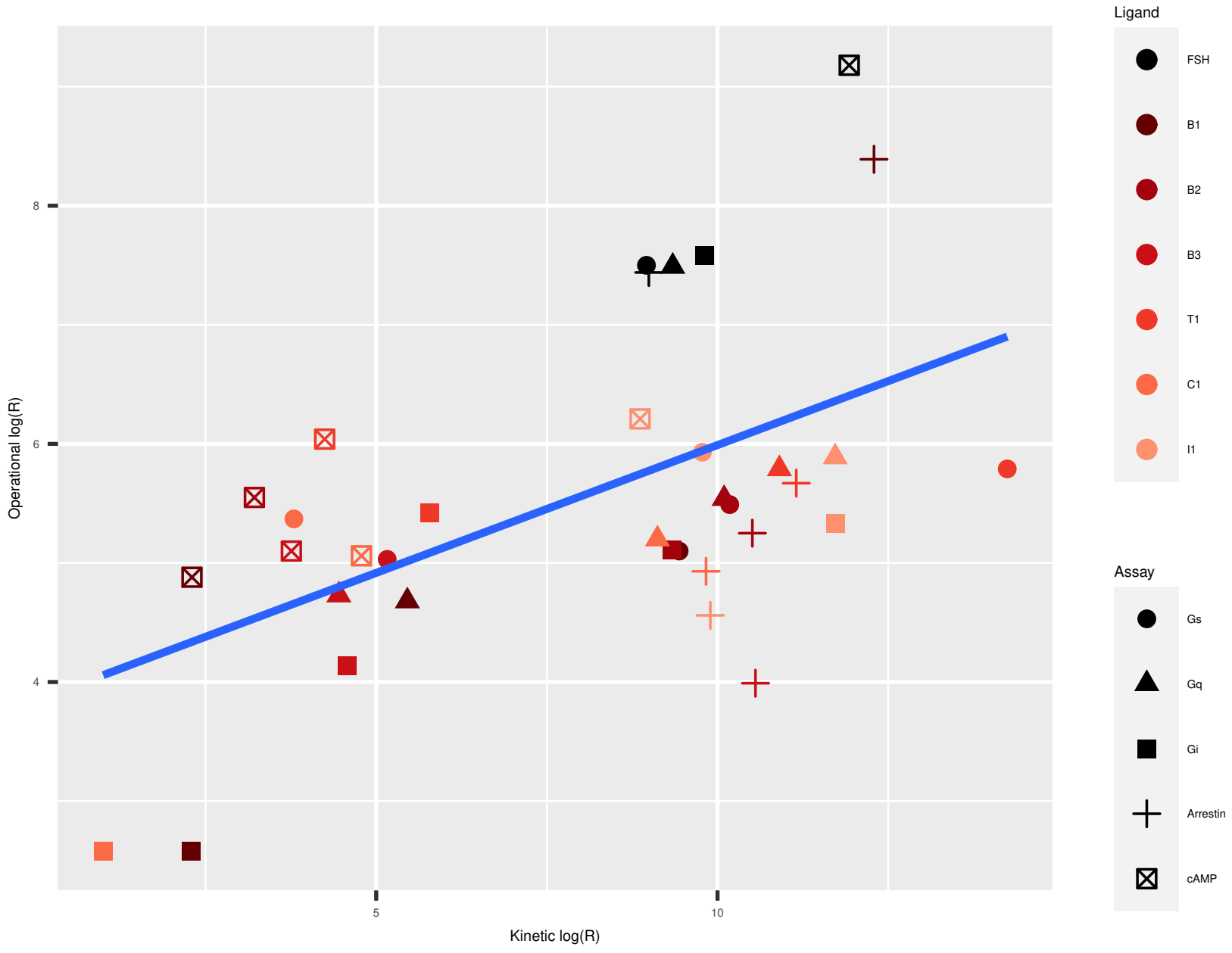


⇒ Large confidence intervals result from ”incomplete/noisy” time series.


"FSH" cluster
Higher affinity
smaller "kinetic efficacy"
FSH
T1
B2
B3
B1
C1
I1
Binding
Gs
Gq
Gi
Arrestin
cAMP
Koff
KD
Kon
Direction
-5.0 -2.5 0.0 2.5 5.0
Value (logscale)
Ligand
FSH
T1
B2
B3
B1
C1
I1


"FSH" cluster
Higher affinity
smaller "kinetic efficacy"
FSH
T1
B2
B3
B1
C1
I1
Koff
KD
Kon
Binding
Gs
Gq
Gi
Arrestin
cAMP
Direction
Value (logscale)
-5.0 -2.5 0.0 2.5 5.0
HEK293/WT
PC1 (52.48 %)
PC2 (38.96 %)
Ligand
FSH
T1
B2
B3
B1
C1
I1

Consistency with the Operational model



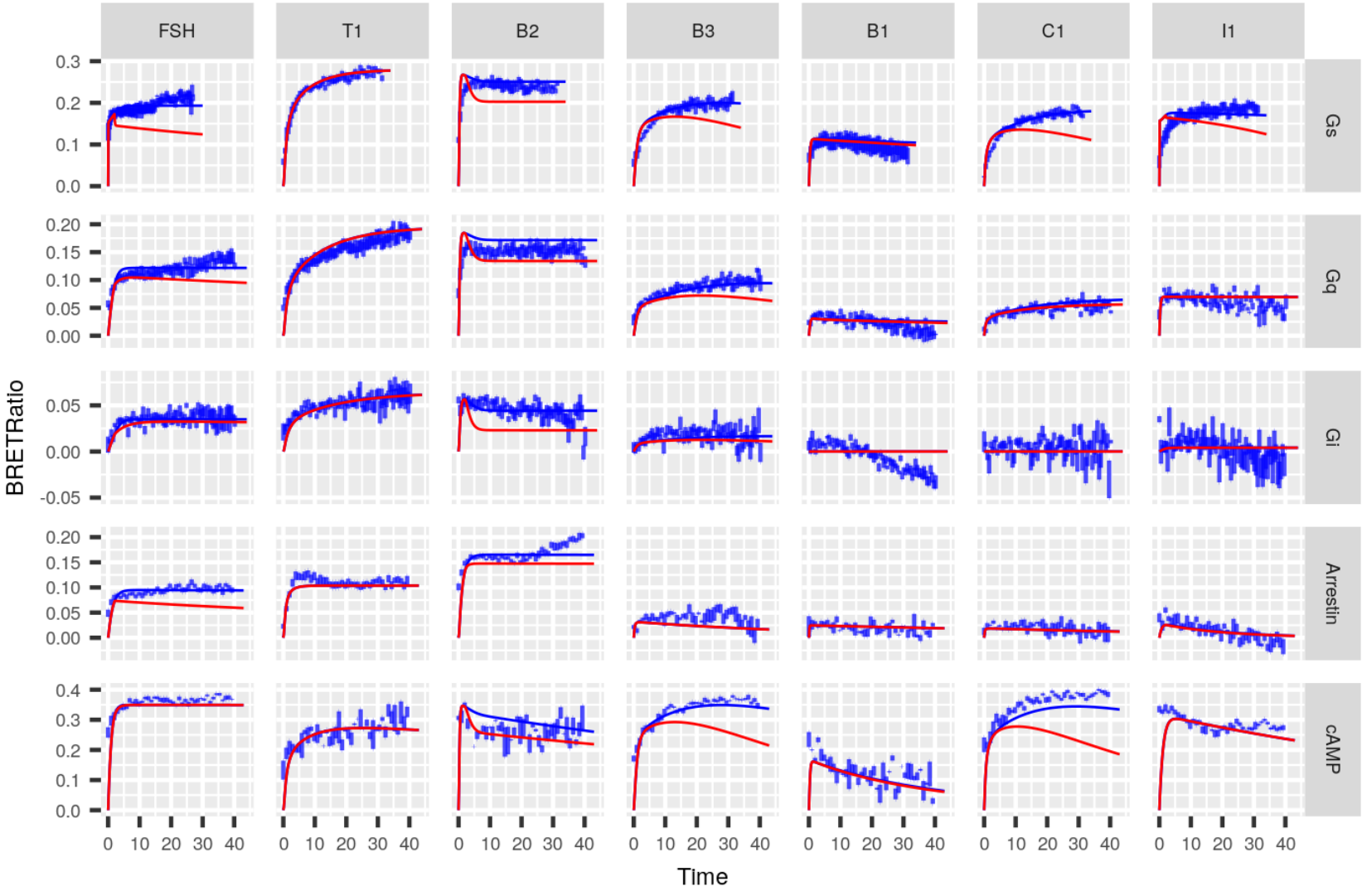
Summary

- We gave a fully kinetic and mechanistic description of Ligand biased, which rely on kinetic data and dynamic (ODE) modeling, with (advanced) statistical parameter estimation and L^1 penalization to reduce combinatorial complexity.
- Our approach was consistent with equilibrium operational model, yet shed lights on ad-hoc clustering analysis.
- Parameter identifiability requires a case by case study (highly dependent on data "quality").

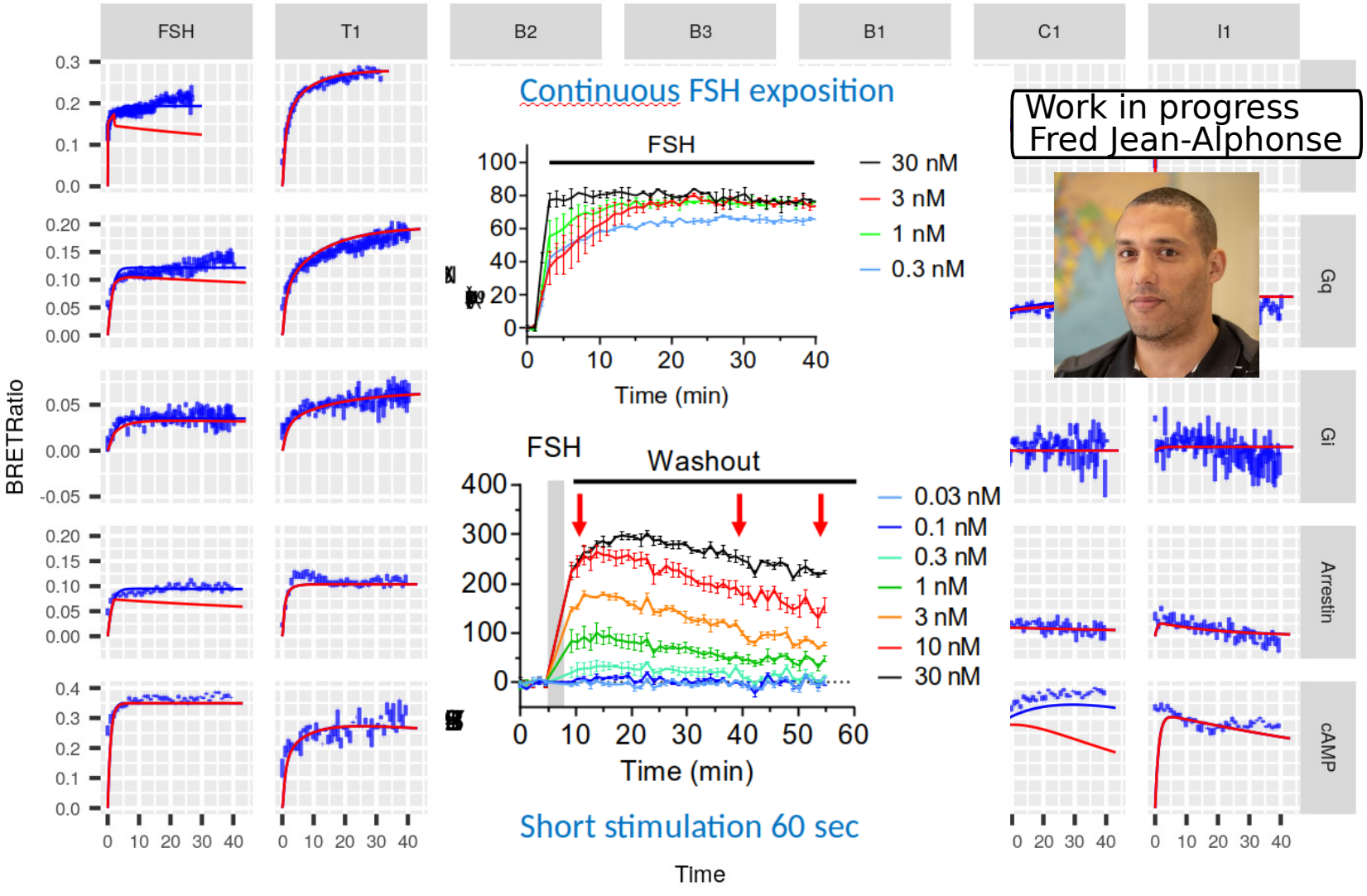
Perspectives

- Include different cell-type -> system bias
- Measurements : sensor needs to be robust ! (Initial rate, long time behavior, dynamics range)
- Use time-dependent input to improve identifiability

Prediction for washed out experiments



Prediction for washed out experiments



Thanks for your attention !

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M. Ayoub et al., Molecular and Cellular Endocrinology 436 (2016)

L. Riccetti et al., Scientific Reports 7 :940 (2017)

R.Y. et al., Methods in Molecular Biology, in press (2018)

De Pascali, ..., R.Y,..., *in preparation*

Fitting and identifiability : why not more relevant details ?

General trends while increasing model complexity :

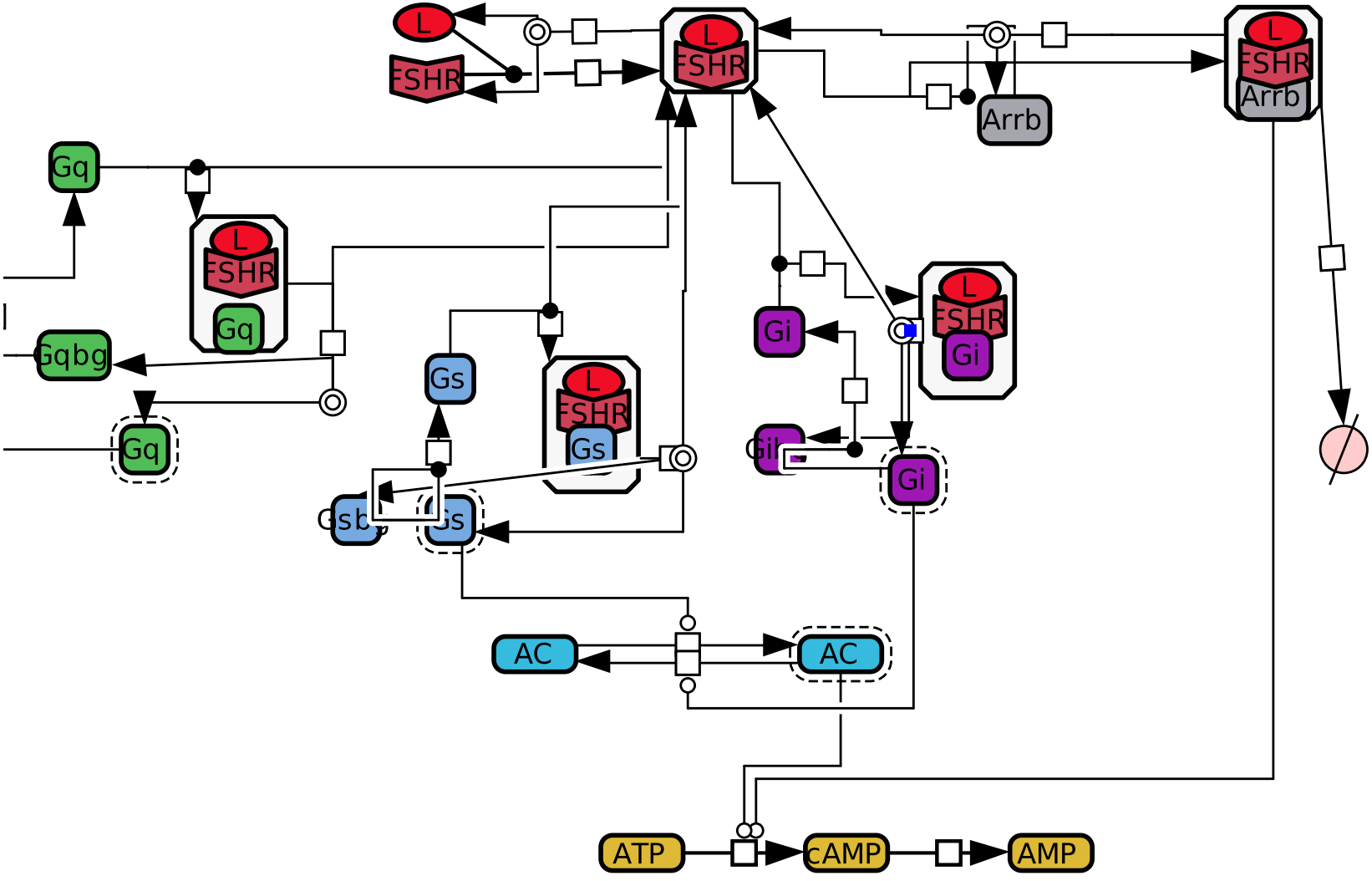
- Improve data adjustement (increase likelihood)
- Loss of parameter identifiability

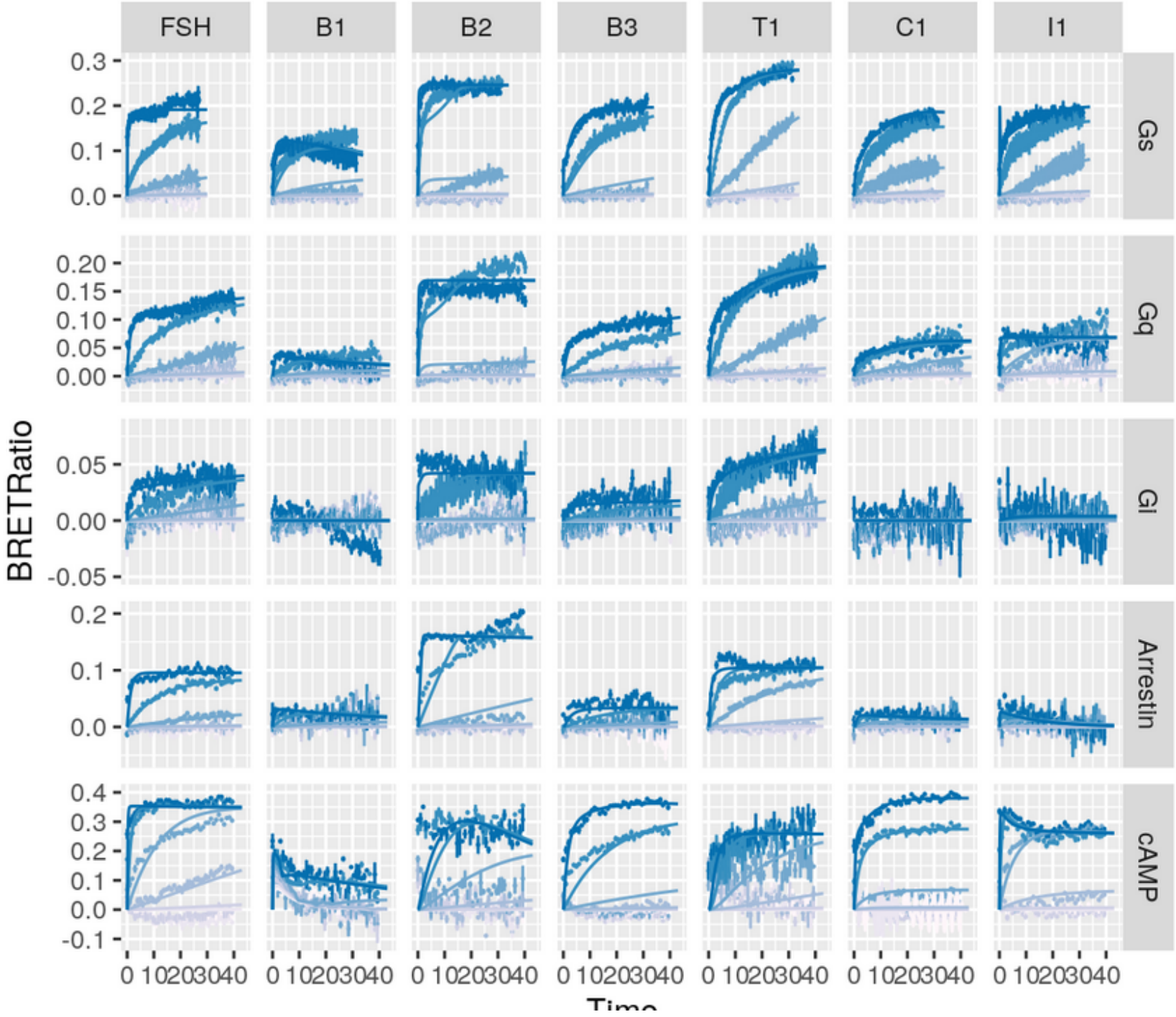
$\Rightarrow$ Model selection provides a solution to find an optimum model within a series of submodel, and given a dataset.

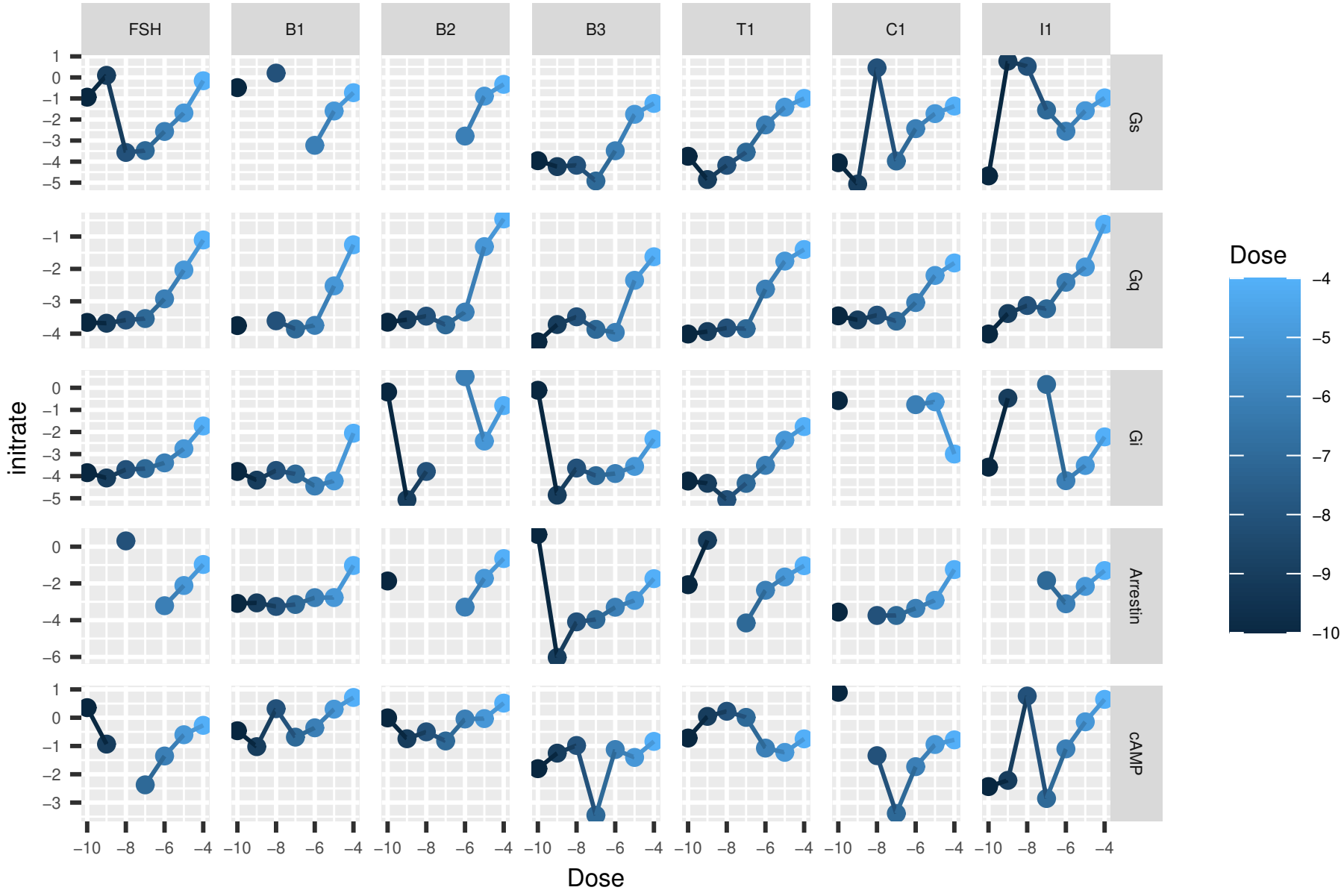
Methodological challenges

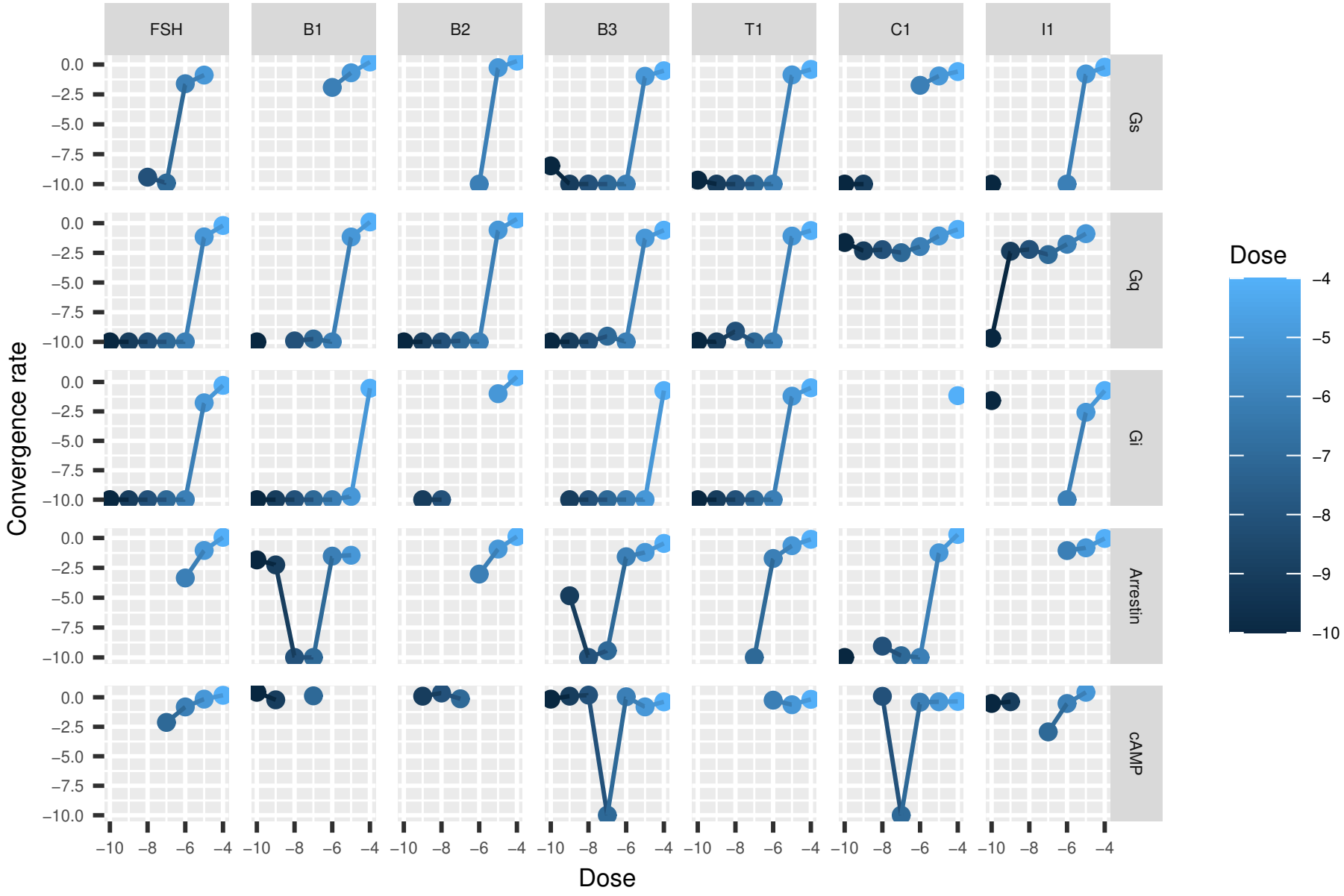
$\Rightarrow$ How to make the network and parameter inference more robust ?

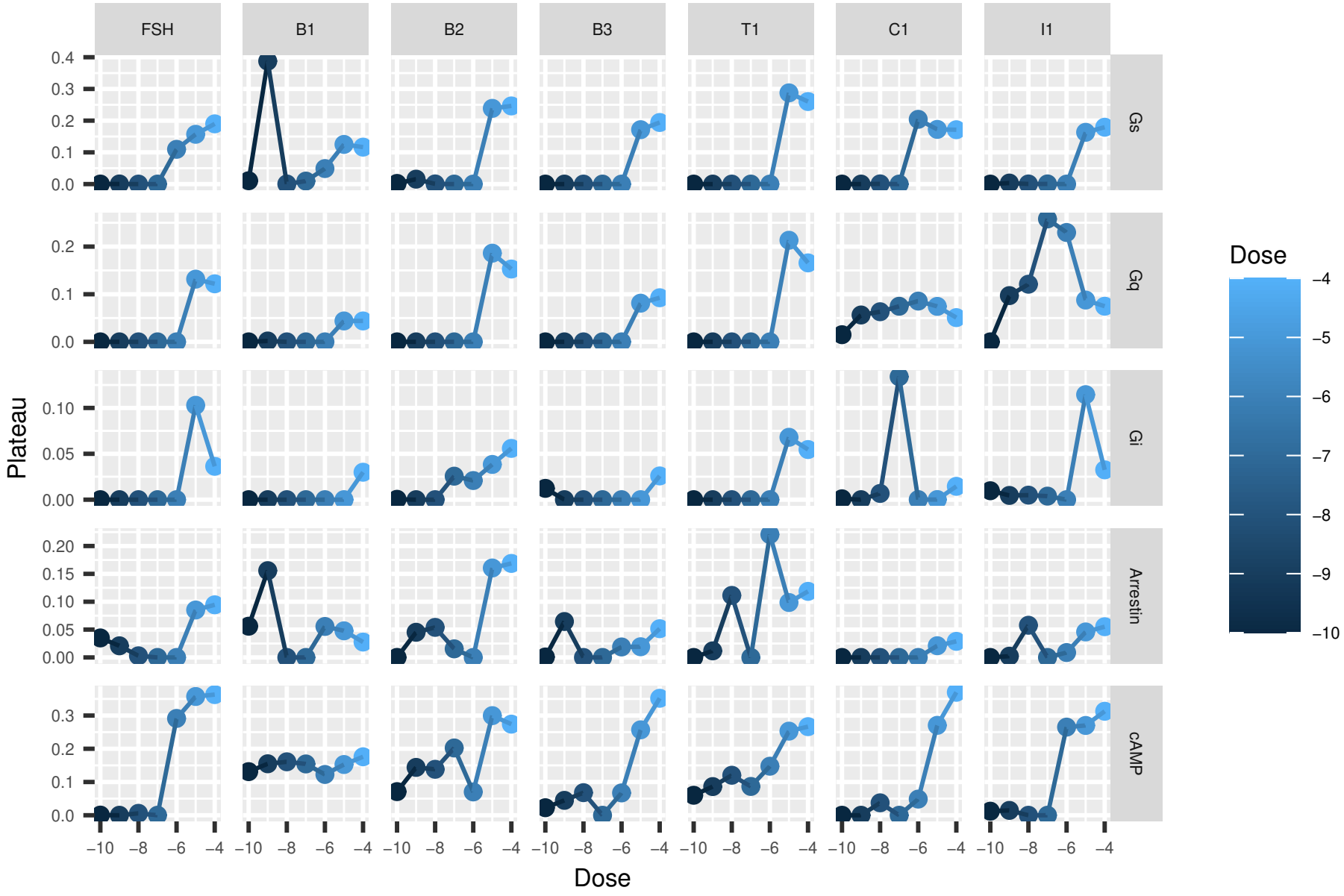
$\Rightarrow$ How to make the modeling and optimization process "automatic" and "generic" ?

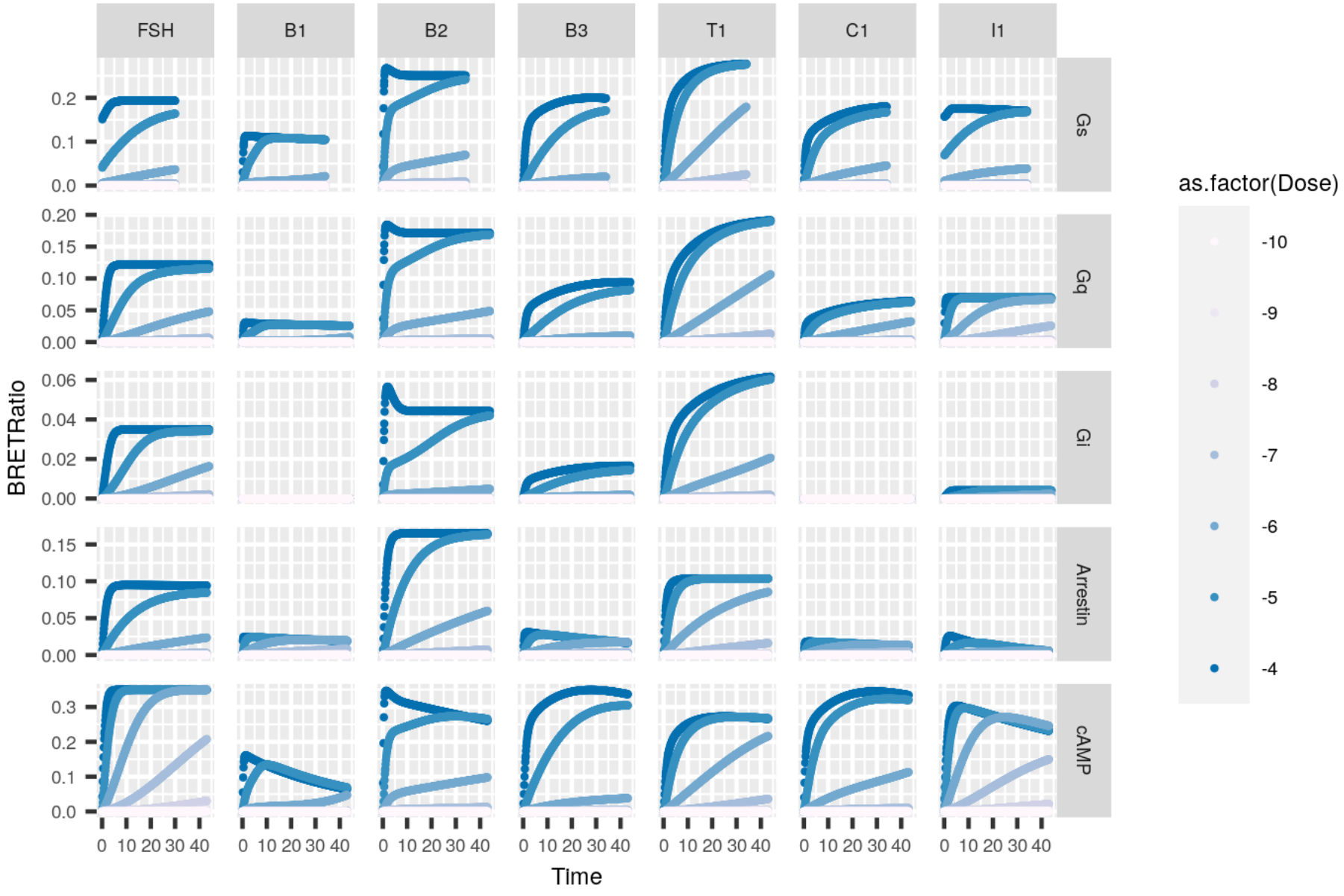

L
FSHR
L
FSHR
Arrb
L
FSHR
Arrb
Gq
L
FSHR
Gq
Gqbg
Gq
Gs
L
FSHR
Gs
Gi
L
FSHR
Gi
Gi
Gsb
Gs
AC
AC
ATP
cAMP
AMP


FSH
B1
B2
B3
T1
C1
I1
Gs
Gq
Gi
Arrestin
cAMP
BRETRatio
0.3
0.2
0.1
0.0
0.20
0.15
0.10
0.05
0.00
0.05
0.00
-0.05
0.2
0.1
0.0
0.4
0.3
0.2
0.1
0.0
-0.1
0 10203040
Time


FSH
B1
B2
B3
T1
C1
I1
Gs
Gq
Gi
Arrestin
cAMP
initrate
Dose
Dose
−4
−5
−6
−7
−8
−9
−10


FSH
B1
B2
B3
T1
C1
I1
Gs
Gq
Gi
Arrestin
cAMP
Convergence rate
0.0
−2.5
−5.0
−7.5
−10.0
−10
−8
−6
−4
Dose
Dose
−4
−5
−6
−7
−8
−9
−10


FSH
B1
B2
B3
T1
C1
I1
Gs
Gq
Gi
Arrestin
cAMP
Plateau
Dose
Dose
−4
−5
−6
−7
−8
−9
−10


FSH
B1
B2
B3
T1
C1
I1
Gs
Gq
Gi
Arrestin
cAMP
BRETRatio
Time
0 10 20 30 40
as.factor(Dose)
-10
-9
-8
-7
-6
-5
-4